THE NATURE OF ORGANIC COLOR IN WATER

By

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I. INTRODUCTION

Waters containing color resulting from their natural environment are found throughout most parts of the world. While organic color is most frequently found in surface waters, it is occasionally found in shallow or deep wells in limestone regions where solution topography prevails.

The United States Public Health Service (1) has set a maximum limit for drinking waters of 15 color units on a platinum-cobalt scale. This limit has been imposed mainly for aesthetic reasons as the materials responsible for color in water are not known to be physiologically harmful. In practice, most municipal treatment plants strive to maintain finished waters with color values of 10 or less.

Almost all of the research to date involving naturally colored water has been concerned either with empirical methods of treatment for color removal or with the mechanism of color coagulation. Little attention has been directed to the fundamental nature of the organic materials that are responsible for color in water. Therefore, the primary purpose of this investigation is to study various physical and chemical properties of organic color in water, in the hope that such basic information may eventually lead to a more complete understanding of treatment processes.

Because of the widespread occurrence of this type of water, it is an additional objective of this research to determine the uniformity of these properties in colored waters from a variety of sources.

II. HISTORICAL

Nature of Organic Matter in Water

Aschan (2) carried out the first extensive research on these substances with an analysis of six Finnish lake and river waters. The humus material was precipitated with $FeCl_3$ and subjected to ultimate analysis with the following results: carbon content was found to vary from 44.99 to 54.10 per cent, hydrogen 3.86 to 5.05 per cent, nitrogen 1.46 to 4.23 per cent and oxygen from 38.76 to 47.93 per cent. He concluded that these materials were acids of strength comparable to phenol.

Birge and Juday (3) observed that the carbon/nitrogen ratio was larger in more highly colored lakes, a fact which has led many authors to regard nitrogen as an impurity.

Saville (4) reported from the results of some qualitative electrophoretic experiments that organic color is present in water as a negatively charged colloid. Behrman, Kean and Gustafson (5) found that little color in water would dialize through a parchment membrane, indicating a colloidal nature. These authors also noted the indicator action of colored water and that most of the color could be oxidized by chlorine.

Christman (6) determined the rate of migration in cm/sec/volt of organic color bodies in 32 natural waters and of two aqueous dye solutions in an electrophoresis cell. All colloids present in the 32 waters were found to be negatively charged.

In 1910, Dienert (7) detected fluorescent materials present in natural waters and noted that this property was lost by mild oxidation.

The use of absorption spectra analyses for the investigation and routine checking of contaminated surface water was first described in 1936 by Demmering (8). This author found the absorption bands produced by lignin substance offered a means of checking changes in the concentration of organic material. Demmering applied this as a criterion for amount of chemicals to be used for chemical treatment.

Datsko (9) carried out photometric investigations of color reactions of sulfuric acid with organic substances in natural waters. He observed an intense coloration produced in the reaction and noted that the intensity followed Beer's law. No description was furnished as to the manner of preparation of his humic acid.

Keiser (10) has reported a differentiation of organic substances in natural water. According to this author alum removes more of the oxygen oxidizable material than the chlorine oxidizable material.

More recently, Flaig and Otto (11) have carried out investigations of humic materials and model substances on plant life. These authors added polyhydroxyanthraquinone to spring water at a concentration of 10^{-5} gms/ml and found that the plants grew normally, using this material precisely as plants use natural humic acid.

Goryunova (12) has investigated the character of dissolved organic substances in water of Glubokoe Lake, Russia. The water of this lake was found to contain 96 gms/m^3 of organic matter, the bulk of this material being made up of high molecular weight fatty acids and colloidal complexes. He also noted small amounts of substances that would reduce Fehling's solution.

According to Isachenko and Egorova (13) the earthy smells in reservoirs are caused by actinomycetes. Odors of this type have been noted frequently in concentrates of organic colored water.

In 1954 Ponomareva and Ettinger (14) obtained dark, easily precipitated substances of the humic-ulmic acid type from organic matter in waters of the Neva River. This material was found to comprise only 5 to 6 per cent of the total organic matter. In addition, apocrenic acid concentrations were found to reach the same percentage. The remainder of the organic material was thought to consist of lightly oxidized fractions of crenic acids.

Goryunova (15) has isolated and identified 50 per cent of the organic matter in the water of Beloe Lake, Russia. This author concluded these are humus materials but fatty acids and polysaccharides are also present.

Skopintsev and Krylova (16) have stated that aqueous humus of terrigenous origin is responsible for the yellow color of water. These authors found a linear relation between color intensity determined colorimetrically and the coefficient of extinction determined spectrophotometrically. No such relation was found to exist between the total organic content and either color intensity or extinction coefficient.

Raudnitz (17) isolated a water soluble, surface-active phosphoric ester of humic acid from rhododendron leaves. Subsequent hydrolysis yielded a uniform humic acid free of nitrogen and sulfur, insoluble in common organic solvents but soluble in pyridine and aniline. Infrared data showed the presence of hydroxyl and carbonyl groups.

Wilson (18) stated that colored organic materials in water are fulvic acids, a fraction of natural soil humus. The author believed no

specific chemical test for these materials will be developed because of the diversity of functional groups present, but a quantitative estimation of these acids can be obtained from their ultraviolet absorption spectra. Using a standard solution of fulvic acids from phragmites peat, Wilson observed that for a given concentration absorption at 300 mμ was constant in the pH range 1.0 to 5.0 increasing over pH 6.0. Application of this technique to several natural waters led to the following conclusions (a) All samples contained appreciable quantities of fulvic acids. (b) All absorption spectra were similar. (c) Since fulvic acid is the most water soluble fraction of natural soil humus it would be expected to be found in natural waters in higher concentrations than either humic or hymatomelanic acids.

Fouling of anion exchange resins by organic materials in colored water is a problem of some industrial significance. Numerous authors have observed organic fouling of strongly basic anion exchange resins of the quaternary types I and II and have ascribed the differences in their fouling rates to the structural differences in their quaternary ammonium substituents. The foulants are held on the resins rather strongly and are not removed by normal regeneration. Excellent reviews of this problem are provided by Frisch et al. (19) and Frisch and Kunin (20). In addition, Ungar (21) has reported fouling of weakly basic anion exchangers and that resins of this type exhibit a limited exchange capacity for humic acids.

One of the most recent publications in this area is an extensive investigation of the yellow organic acids of lake water carried out by Shapiro (22). This author has outlined a separation scheme for the isolation of a "free acid" fraction of the organic materials present in

the water. Data obtained by infra-red and ultraviolet spectroscopy and by various organic spot reactions led this author to report that these acids are aliphatic unsaturated polyhydroxy dicarboxylic acids with an approximate molecular weight of 456. No evidence was found in this work of the existence of aromatic rings and dialysis studies indicated a lack of colloidal character. Shapiro suggests the name "humolimnic" acids for these materials to indicate their probable relation but improbable equivalence with natural humic acids of the type found in soils.

The Nature of Organic Material in Soils

Because of the relation of these coloring substances to the organic matter present in soils, it is necessary to review the work in this area of chemistry. No attempt will be made to critically outline the early developments in this field as this has been done by Waksman (23). However, the major findings will be discussed particularly where there is an appreciable correlation with data obtained on the organic materials in water systems.

The term humus dates back to the Roman era when it was generally applied to the complete soil substance. From this time to around the 17th century little was known about the nature or functions of soil. At the start of the 18th century Linnaeus classified soils in a manner similar to his classification of plants, humus daedalea (garden soil), humus ruralis (field soil), etc. In the latter half of the 18th century, humus began to be considered in terms of decaying organic matter. However, the general ideas concerning the chemical nature of humus were very vague.

Unfortunately, the term humus came into general use when organic chemistry was in its infancy and was not always used to designate the same organic materials. This was responsible for some degree of confusion among workers in the field of soil science, a situation which has never been entirely remedied.

Perhaps the earliest scientific classifications of humus are those of Oden (24) and Ramann (25). Ramann regarded humus as "colloidal complexes of varying composition, consisting of unchanged colloids of the original plant substance mixed with carbon-rich decomposition products." Ramann recognized the importance of micro-organisms in the synthesis of humus, a fact disregarded by Oden. The latter regarded humus as "those dark colored bodies of unknown constitution which originate in nature through the decomposition of organic substances...; they possess a definite affinity for water and show, if not true solution, a distinct swelling."

Oden also supplied the first generally accepted system of naming the four common fractions or preparations of soil organic matter. Up until this time there were in common use 43 different names for the four preparations.

Oden's classification scheme is shown in Table 1.

In 1930 this system was modified by Page (26), and resulted in the most widely used classification system to date. He suggested the name "humin" for preparation 4 and "humic acid" for preparation 2.

The multitude of names and classification systems during this early period was due to a large extent to the variety of sources from which the humus materials were obtained. The early theories about the simplicity and uniformity of the substances in soil were gradually

TABLE 1

Classical Fractions of Soil Organic Matter

Preparation	Name
1. Soluble in alkali, not precipitated by acid	Fulvic Acid
2. Soluble in alkali, precipitated by HCl, insoluble in alcohol	Humus Acid
3. Soluble in alkali, precipitated by HCl, soluble in alcohol	Hymatomelanic Acid
4. Insoluble in alkali	Humus Coal

replaced by the 20th century theories that the composition of humus material and to some extent the properties, were dependent on the source, manner of extraction, and length of formation time of the organic substances.

One of the earliest and perhaps the most persistent theory of the origin of humus was that it was produced by the action of mineral acids on sugars. This trend in research prompted many investigators to produce artificial humus materials in the belief that these were formed along the same pathways that nature employs in producing humus. Boullay (27) calculated the chemical constitution of an artificial preparation as $C_{30}H_{30}O_{15}$. Malaguti (28) suggested that HNO_3 changes sugars into oxalic acid whereas HCl and H_2SO_4 give humus matter. Under the influence of acid, the sugar loses water giving first a compound of the structure $C_{12}H_{28}O_{14}$ which changes to humic acid. The mechanism was as follows:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \longrightarrow \underset{\text{Humic acid}}{C_{12}H_{12}O_6} + 5\ H_2O$$

Stein (29) suggested the formula $C_{24}H_{18}O_9$ for the same material and Berzellius (30) adopted yet another formula, namely $C_{32}H_{32}O_{16}$ for this preparation.

Berzellius was one of the first workers to isolate humus material from a natural water. He believed these compounds were also present in plants and soils. He suggested the name crenic acid for his preparation which he believed could be oxidized to apocrenic acid by the action of air. These preparations had formulas of $C_{24}H_{12}O_{16}$ and $C_{24}H_6O_{12}$, respectively. In addition he noted the salts of these acids with alkalies. Magnesium and iron were soluble in water and could be transported from soil to water in this manner.

A most extensive study of dark colored compounds extracted from soil, peat, coal and prepared artifically in the laboratory was made by Hermann (31). His system of classification only added to the complexity of the problem but he did note the precipitative effect of heavy metal acetates on these substances and the fact that although most humus materials contain nitrogen, many do not.

Around the turn of the century investigators began to doubt the validity of explaining natural processes of humus formation by results obtained from artificial materials produced by treatment of sugars and other complex organic substances. Attention turned towards investigation of the physical and chemical properties of the natural humus. Thus, Sestini (32) demonstrated in the humic complex the presence of ethereal and anhydride substances, as well as hydroxyl, alkyl and ketonic groups.

The recognition of the mixed and indefinite nature of the various humic acids and the turning of attention towards the elucidation of the complex chemical composition of the natural materials was the most important advance in the history of research in this area.

Van Bemmelen (33) was one of the first researchers to investigate the colloidal properties of soil organic matter. He believed that the humus complexes were amorphous and colloidal in nature and originated from the colloidal substances in plant life.

Hoppe-Seyler (34) investigated both natural and artificial products and found that treatment with alkali gave dark solutions in the presence of air, but acid treatment gave the same result even in a hydrogen atmosphere. Included in his organic compounds were pyrogallol, pyrocatechuic acid, pyrocatechin, and quinone, which all gave similar humic materials on oxidation. He noted xylan, or wood gum, in soil in an unchanged state, and found that lignin complexes take an active part in the formation of humic acids.

Miklauz (35) pointed out that the compositions of the alkali and alcohol soluble fractions are affected by the strength of extractment and the extracting temperature; and that the highest carbon content is usually found in the alcohol soluble fraction.

At this time a new concept appeared which has been called the colloid-chemical approach, mostly due to the outstanding works of Van Bemmelen, Baumann and Gully (36). This new trend was based upon two main ideas. The first was that the acidity of humus is not due to free humic acids but is associated with the reactions of humus with mineral salts, bases being adsorbed on the colloidal surfaces of the organic matter releasing mineral acids. Second, these workers concluded from all existing data on natural and artificial preparations that humic acids are not definite chemical compounds but mixtures of plants and animal residues, partly decomposed, partly conserved because of resistance to decomposition, and combined in a colloidal state. The possibility that

pure organic compounds are present in the complex was not excluded but such a compound as humic acid was believed to be entirely absent from soils.

Baumann and Gully later reported the electrical conductivity of humic acid to be much less than that of a 0.5 per cent solution of acetic acid and took this as proof of a lack of free acids.

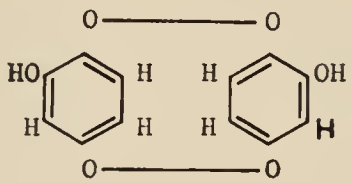
In 1907-1913, Schreiner and Shorey (37) reported what seemed to be conclusive proof of the absence of single humic acid structure when they reported isolating from the humic and the hymatomelanic acid fractions of soil several pure organic compounds. Among these were vanillin, salicylic aldehyde, 2-hydroxy- and dihydroxystearic acid, trithiobenzaldehydes, and various resin acids. The amounts of materials extracted (50 mg of dihydroxystearic acid from 25 Kg soil) were very minute and for this reason failed to convince many workers in the field.

Interest in humic acid as a pure substance persisted as the works of Maillard (38), Samuelly (39) and Gortner (40) postulated humic acid as a complex of sugars and proteins.

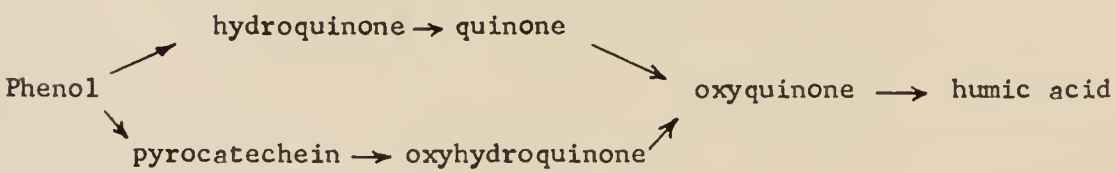
Oden (41) showed by potentiometric titrations that organic acid ions are present in soils and peats. He attributed the low pH of water solutions or dispersions of these materials to a release of hydrogen ions from an acid group rather than as a surface phenomenon. Oden first reported that these acids were tribasic and later tetrabasic with an equivalent weight of 339. As had other authors before him, Oden recognized the tendency of these substances to readily yield colloidal dispersions in water.

Oden proposed the formula $C_{60}H_{52}O_{24}(COOH)_4$ of molecular weight 1332. He suggested that hymatomelanic acid is a hydrolysis product of

humic acid and forms only during the extraction of humic acid itself. He reported an equivalent weight of 250 for hymatomelanic acid and noted that it has a higher carbon content. Because he obtained decreasing nitrogen content in successive purification procedures he concluded that the nitrogen in humic and hymatomelanic acids is an impurity. One of the first authors to quantitatively determine functional groups in these organic molecules was Eller (42), who concluded from his data on the relative amounts of carbonyl oxygen in the preparation that the natural products had little relation to the products obtained by the action of mineral acids on sugars. Leopold (43) agreed with this theory but found that, on oxidation of certain phenolic substances, products were obtained which were similar in physical and chemical properties to the natural humic acids. Leopold's artificial products prepared by the oxidation of phenol, quinone, and hydroquinone in alkaline solutions contained 58.05 per cent carbon of the formula $x(C_6H_4O_3)_2$ with a basic structure of,



The process of oxidation was outlined as follows:



His natural humic acid contained 59.6 to 60.2 per cent carbon, 3.2 per cent hydrogen and 1.7 to 2.0 per cent nitrogen, which was

considered as an impurity. The natural products were considered to arise from the following reaction:

$$C_6H_{12}O_6 + O = C_6H_4O_3 + 4\,H_2O$$

The acidity of these materials was attributed to the phenolic hydroxyl groups.

Fuchs (44) and Leopold (43) demonstrated that humic acids prepared from peat contain both carboxyl and phenolic hydroxyl groups. The carboxyl groups form salts and the hydroxyl groups are capable of combining with bases. These authors prepared salts of humic acid by shaking the acid with alcoholic potassium acetate. The base content of the salt was found to correspond quantitatively to the methoxyl content of the saturated methylated humic acids. The methoxyl content of the natural humic acid was found to vary considerably with the source of the material. Fuchs concluded that humic acid contains three to four hydroxyl groups, three to four carboxyl groups, one methoxyl and one carbonyl group. The hydroxyl groups were assumed to be subject to methylation. He considered humic acids then as "oxy-carbonic acids originating in the decomposition of dead organic material as dark amorphous substances capable of giving off hydrogen ions and of forming salts and possessing base-exchange capacity." The following formulae were suggested for the complexes isolated from peat.

$$C_{49}H_{52}O_{10}(OCH_3)_2(COOH)(CH_3CO)(OH)_2$$

$$C_{53}H_{46}O_{11}(OCH_3)(COOH)(OH)_4$$

$$C_{59}H_{41}O_{17}(COOH)_4(OH)_3(CH_2CO)$$

Fuchs regarded the presence of nitrogen in different humic acids as due to the bonding of a protein molecule to a nitrogen free compound or to the replacement of oxygen by ammonia in the cyclic compound.

Leopold (43) determined the carbonyl number of several humic acid preparations by reacting them with phenylhydrazine hydrochloride. Fehling's solution was employed to decompose excess phenylhydrazine, the amount in excess being measured be determining the amount of evolved N_2. His results varied from 0.22 per cent to 2.31 per cent carbonyl and were at best inconclusive.

Reports of equivalent weights and molecular weights for these acids vary widely and depend on the source of the material. Molecular weight data obtained by Samec and Pirkmaier (45) are shown in Table 2.

TABLE 2

Molecular Weights of Some Humic and Hymatomelanic Acids

Acid	Source		
	Lignite	Peat	Brown Coal
Humic	1445	1235	1345 gms/mole
Hymatomelanic	855	761	739 gms/mole

Plunguian and Hibbert (46) were the first to consider the possibility of an enol-keto tautomerism in the structure of natural humic acid, and ascribed the differences in properties of the natural and artificial preparations to the relative amounts of aromatic and aliphatic hydroxyl groups in each. The natural material was found to have mainly aromatic

hydroxyl while the artificial had a predominance of aliphatic hydroxyl groups.

Fuchs (47) studied the relation between humic acids and lignin and found them to be very similar structurally by testing with various organic spot reactions. He found the products of nitration to be particularly similar and concluded they are isonitrosoketohydroxy-carbonic acids of high molecular weight. According to Fuchs, the difference between the lignin and humic nitro derivatives is the manner of N-linkage in each. Molecular weights (determined in $(CH_3)_2CO$) ranged from 1410 to 1465 gms/mole for methylated nitro derivatives of lignin and 1535 for the corresponding derivative of humic acid.

Fuchs used bromination as proof that a tetrahydrobenzene ring is present in natural humic acid and Fuchs and Stengel (48) were successful in identifying various organic acids and nitrophenols by oxidation of humic acid preparations with HNO_3.

Ludmila (49) has presented evidence that humic acids are cyclic compounds to which are attached carboxyl, hydroxyl, and carboxyl groups and have an average molecular weight of 1400 gms/mole.

Fromel (50) extracted humic acid from coal and peat with NaOH and NaF and proved that the Freundlich adsorption isotherm is followed in the medium concentration range and light absorption in the 2300 to 5000 A^{o} range follows Beer's law.

Ubaldini (51) determined acid numbers of humic acid by suspending humus material in ethanol and titration with alcoholic KOH. Total acid numbers were between 492 and 500 and carboxylic acid numbers between 263 and 267. Methylation with diazomethane showed not all acid groups are

methylated. Carboxyl, phenolic hydroxyl and carbonyl groups were found in a ratio of 8:7:2.

Scheele and Steinke (52) reported molecular weights of humus material as high as 9000, and found a slow decomposition takes place in the presence of alkali giving a water soluble substance that cannot be precipitated by mineral acid.

Galle and Lodzik (53) investigated the functional groups of humus material by reacting model substances with MeOH containing HCl. The acidic MeOH effected an esterification of carboxyl groups and an esterification of phenolic groups. The authors treated phenylmethanol, diphenylmethanol and triphenylmethanol in this manner and found a 90.3 per cent methylation. In model compounds containing both carboxyl and phenolic hydroxyl groups, no distinction was made as to which group was more completely methylated.

Later Scheele (54) used conductometric titrations to show that the equivalent weight of natural humic acid is around 200. Molecular weight determinations by diffusion constants were said to reveal that the molecular weight of humic acid is twice as large in acid solution as in basic solution.

Esh and Guha-Sircar (55) prepared humic acids in the following manner. Soil was washed consecutively with hot alcohol-benzene, water, 2 per cent HCl, and 4 per cent KOH. This filtrate was then precipitated with HCl. Humic acid prepared in this manner was oxidized with H_2O_2 (12 to 15 per cent) in NaOH at 30-40^{o}. Extraction with ethyl acetate gave acidic and phenolic components. Oxidation with chlorine dioxide for 20 hours at room temperature yielded butyric and oxalic acids in small quantities.

Niklas and Genninger (56) also performed extensive studies on the oxidation products of humic acids. They found ferric hydroxide "of a given type and concentration" to act as catalysts in the oxidation of humic acid with H_2O_2. No data were given by the authors but they claim that a gas, a liquid and a solid were obtained in this manner. The gas was described as a pungent, yellow acid which could be condensed to a volatile liquid. A light brown wax was reported as being distilled under pressure at 250° from the residue.

Forsyth (57) subjected humic materials to ultimate and group analyses and arrived at the conclusion that although various humic acids have similar molecular structures, the number and kind of structural groups depends entirely on the conditions and length of time of formation of the humus.

Evstigneev and Nikiforova (58) reported spectral data on hydroxylmethylfurfural obtained from a decomposition of glucose. This material exhibits absorption maxima at 282.5 and 228-230 mμ. Humus substances from sugars were found to show an initial absorption in the visible region increasing rapidly in the ultraviolet.

Kukharenko (59) described a semimicro titrimetric determination of the functional groups in humic preparations. This process involved heating the humic acids at 100°C with calcium acetate. The number of milligram equivalents of NaOH necessary to titrate the AcOH form from the reaction from one gram of sample with calcium acetate equaled the number of carboxyl groups in the humic molecule. No data were reported.

Hadzi (60) also reported spectral data on humic acids. These authors found the infra-red absorption band at 3.25 microns (due to phenolic hydroxyl) was shifted to 3.88 microns when the material was

deuterated (phenolic OD). In addition they found the carbonyl band at 1700 cm^{-1} and stated that the absence of marked absorption bands in the 900 cm^{-1} indicates a lack of acid dimers and proves that the acidic groups in humic acid are mainly phenolic.

Botteri (61) describes lengthy extractions of meladoin, humic acid and lignin. This author concludes on the basis of aromatic substances obtained from alkaline fusion of these materials that there is no similarity in their molecular structure.

Higuchi and Shibuya (62) obtained a reaction product with humic acid in NaOH and the diazonium salt of sulfanilic acid. The purified product was an orange-yellow dye of the following composition: $R(N{:}NC_6H_4SO_3Na\text{-}p)_7$ where R equals

$$C_{106}H_{85}O_{16}(CO_2Na)(OH)_7(CO)_4OMe.$$

Recently a good deal of attention has been focused on the use of model substances for determination of the structural elements in humic materials.

Ploetz (63) produced 1,4-diquinonylbenzene by adding an aqueous solution of the tetrazonium salt of $p\text{-}C_6H_4(NH_2)_2$ to a cooled aqueous or aqueous alcohol solution of quinone. A coupling was noted by the evolution of nitrogen gas. The author concludes the coupling product is an intra-molecular quinhydrone complex, and this same structural type is present in natural humus substance.

Welte et al. (64) used polymers of polyvalent phenols to give X-Ray amorphous humic materials. Auto-oxidation reactions resulted in the formation of oxalic acids, acetic acid and CO_2 by ring cleavage.

Flaig (65) reported that orthoquinones are intermediate products in the formation of humic acids. Substituted hydroxyquinones were used as model substances in investigations on the preliminary stages and decomposition products of humic acids.

The isolation and identification of organic compounds in humus is described by Wedgwood (66). Polycyclic hydrocarbons, pyrene, fluoanthrene, 1,2-benzanthracene, 3,4-benzopyrene, perylene and anthracene have been detected.

Bremmer (67) points out the need for isolative investigation in soil analysis. Extraction of soil organic matter is usually done with alkali, however some research has been done using mineral acid as the extractant. Because, according to this author, acid or base may harm organic matter extractions, new extraction procedures using neutral salts were investigated. These results indicate soil organic humus is intimately tied up with metallic cations. The efficiency of a neutral salt was found to depend on its ability to remove cations as precipitates or soluble complexes. The following order in decreasing efficiency was reported:

oxalate $\rangle$ malonate $\rangle$ salicylate $\rangle$ succinate $\rangle$ adipate $\rangle$
maleate $\rangle$ trichloroacetate $\rangle$ phthalate.

The cations exhibited the following capabilities:

$$Li \rangle Na \rangle NH_4 \rangle K \rangle Mg.$$

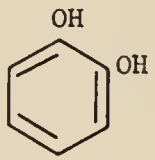
The most effective neutral extractants were found to be those capable of forming complexes with iron.

According to Bremmer there are two distinct theories regarding the origin of humic materials in soils. The first suggests that humus

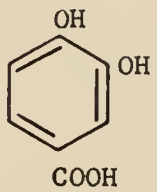
arises from an alteration of plant lignins entering the soil, while the second suggests they are products synthesized by or formed by autolysis of soil micro-organisms. Both of these theories have their proponents among modern soil chemists.

According to Burges (68) all the available evidence suggests that the humic acid fraction is either a single chemical substance or a group of very similar substances. He believes humic acid is primarily non-nitrogenous and nitrogen present in many preparations is due to a secondary combination with protein or amino acids, the main structural element being the quinone group.

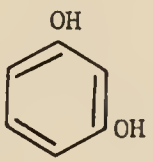
Steelink et al. (69) studied alkaline degradation products of soil humic acid and isolated catechol (I), protocatechuic acid (II) and resorcinol (III) in a mixture from a KOH fusion.



I



II



III

Felbeck (70) detected about 60 components by gas chromatographic separation of pyrolysis products of soil organic matter. The pyrolysis was carried out at a reaction temperature of 300°C and in a H_2 stream, to reduce polymerization of phenolic materials. These pyrolysis products were ether soluble and were further separated by extraction of the ether with aqueous alkali. Roughly 20 per cent of the total organic matter charged to the furnace was accounted for in the product mixture.

Steelink et al. (71) have reported the presence of at least two free radical species in natural humic acid from data obtained in electron paramagnetic resonance spectra. The authors believed one could be a semiquinone radical and the other a quinhydrone-type radical.

III. EXPERIMENTAL PROCEDURES

Collection and Concentration Techniques

The colored water samples secured for this study were collected in five-gallon polyethylene bags or drums with the exception of the one local water which was collected in five-gallon polyethylene-lined cans. Delhez (72) has reported that water stored in polyethylene bottles for several days may show absorption in the ultraviolet due to a continuous release of organic matter from the polyethylene. Accordingly, distilled water samples were stored in each type of container used in the work for periods up to three months. No spurious absorption was noted in any sample from 200 to 300 mμ.

The drums were shipped by truck to the State Boards of Health of the states of Massachusetts, Connecticut, Virginia, North Carolina and Wisconsin for sample collection and returned to this laboratory in the same manner. Numerous samples from the states of Florida and Georgia were collected by the investigator or members of the laboratory staff.

Because the material responsible for the coloration of water is present in minute quantities in the stream, concentration of the organic materials was necessary. Although recovery of the colored materials was possible by several techniques, only two were used in this study, namely vacuum distillation and freeze concentration. Gross solid matter was removed from all water samples prior to concentration by either technique by filtering through a suitable grade of filter paper.

All concentrated samples were swept with nitrogen and stored in pyrex containers at 25°C ± 2° until use.

The distillation apparatus was a laboratory batch-type evaporator manufactured by Precision Scientific Co., Chicago, Ill. The vacuum source was an ordinary water aspirator which provided an internal pressure of 79 mm Hg at 45°C. The distillation was carried out between 45 and 50°C to lessen any possibility of decomposition or combination of chemical species by oxidation or other means. In a usual concentrating run, 40 liters of water was concentrated to a final volume of 1.0 liter in six hours of continuous operation.

Freeze concentration was carried out in a 16 ft^3 Crosbey home freezer, fitted with a 25-gallon polyethylene drum and an electrical stirrer. In normal operation, 20 gallons of stream water was concentrated to a final volume of 1.0 liter in approximately 110 hours of continuous operation.

Instrumental Methods

All ultraviolet absorption spectra were obtained on a Carey Model 14 recording spectrophotometer from 200-800 mμ at a scanning speed of 25 Å/sec, using 1.0 cm quartz cells.

Infra-red absorption spectra were determined on a Perkin-Elmer Model 137 Infracord. Solid samples were run by the potassium bromide technique while liquids were determined on sodium chloride plates.

Fluorescence spectra were obtained with a fluorimeter assembled at the University of Florida. It consisted of a converted Perkin-Elmer Model 12 B monochromator fitted with a lithium fluoride prism and a

55 watt Osram mercury lamp. The detector, an IP 21 photomultiplier tube, was situated at an angle of 90° from the incident beam and was connected to an El Dorado Model RH 200 photometer and a Varian Model G-11-A recorder. A color filter that transmitted the 365, 405 and 436 m mercury lines was placed between the source and the sample.

All color values were determined on a Lumetron Model 450 filter photometer, a product of Photovolt Corporation, New York, New York. The instrument was fitted with a blue filter having maximum transmission at 420 mμ and provided a 15 cm light path. The reference standard for color tests, according to the procedure recommended by the APHA for the sanitary examination of water, is a solution of 1.246 gms potassium chloroplatinate and 1.0 gm crystallized cobaltous chloride in 100 ml concentrated hydrochloric acid, made up to 1.0 liter with distilled water. This solution has an assigned color value of 500 color units. A standard color curve was obtained by measuring the optical densities of appropriate dilutions of the color standard on the Lumetron at 420 m . It was necessary to correct the optical density values for turbidity present in the color samples. The general procedure described in the Lumetron manual (73) was employed.

All pH values were determined on a Beckman Model G pH meter with glass and saturated calomel electrodes. The potentiometric titrations in nonaqueous solvents were carried out with the same titrimeter equipped with platinum and saturated calomel electrodes.

Light scattering data were obtained with a Series 1965 Brice-Phoenix Light Scattering Photometer. A General Electric D. C. galvanometer with a sensitivity of 0.0015 μa/mm was used with this instrument.

A Welsbach Type T-23 Laboratory Ozonator was used to supply the gaseous ozone required in the oxidation experiments. Operated at 7 psig and 100 VAC, this instrument produced 80 mg/l O_3 in an oxygen stream at a flow rate of 0.03 S.C.F.M.

Electrophoretic mobility data were obtained with a Briggs microelectrophoresis cell using the technique and accessory apparatus described by Pilipovich et al. (74) and Black and Smith (75).

Methylation Procedure

The methylating agent used throughout this study was diazomethane and was prepared in the following manner.

A 125 ml distilling flask was fitted with a condenser and a long stem dropping funnel. To the flask was added 6 gms of potassium hydroxide dissolved in 10 ml of water, 35 ml of methanol, 10 ml of sodium-dried ether and a teflon stirring bar. A solution of 21.5 gms (0.1 mole) of p-tolylsulfonyl-methylnitrosamide (diazalid) dissolved in 125 ml of ether was added to the dropping funnel. The distilling flask was stirred in a water bath at 70-75°C, while the contents of the funnel was added at such a rate that the 125 ml volume was delivered in 15 to 20 minutes. The distillation was continued until the distillate was colorless. The usual yield was 2.7 to 2.9 gms of diazomethane.

The diazomethane was standardized by reacting an aliquot with a weighed excess of pure benzoic acid in absolute ether. The excess benzoic acid was titrated with standardized sodium hydroxide to a phenolphthalein endpoint with rapid stirring.

The procedure for the quantitative methylation of the fulvic acids and model compounds was as follows: A weighed sample was placed in an excess of standardized diazomethane solution and allowed to stand in the cold for 48 hours, or for such time as was necessary to achieve maximum methylation. At this time, a weighed excess of benzoic acid was added to react with the remaining diazomethane and the excess titrated with standard aqueous alkali. From these data it was possible to calculate the moles of CH_2N_2 consumed per mole of sample, or, in the case of the fulvic acids, per unit weight of sample. The latter value is hereafter referred to as $\bar{n}$. That is

$$\bar{n} = \frac{\text{moles } CH_2N_2 \text{ reacted}}{100 \text{ gms fulvic acid}} ,$$

and if it is assumed that both the carboxylic acid and phenolic hydroxyl groups are subject to methylation, and that these same groups constitute the titratable acidity,

$$\bar{n} = \frac{\text{number of acid groups (-COOH and } \phi\text{-OH)}}{100 \text{ gms fulvic acid}} .$$

IV. EXPERIMENTAL RESULTS

Characteristics of Raw Waters

As mentioned in Chapter III, all water samples were passed through a roughing filter before concentration. Several qualitative filters were tried and no measurable effect on color or pH of the samples was noted. Centrifugation at 30,000 rpm also produced no loss in color or change in pH. Prolonged storage of colored water has only a slight effect on the color value, as indicated by the following experiment with water A. Approximately 2.5 liters was swept with nitrogen and stored in a cold room. The data in Table 3 show only a slight loss in color over a six-month period.

TABLE 3

Effect of Storage on Color in a Nitrogen Atmosphere

Storage time (weeks)	$O.D._b$ (x 10)	$O.D._r$ (x 10)	Color
0	6.4	0.75	204
1	6.0	0.70	188
4	6.0	0.70	188
8	6.1	0.70	192
16	6.0	0.71	188
24	6.1	0.68	188

In the presence of oxygen, however, the color was not quite as stable. Approximately the same volume of water A was swept with oxygen and stored in the same manner, with the results as listed in Table 4.

TABLE 4

Effect of Storage on Color in an Oxygen Atmosphere

Storage time (weeks)	$O.D._b$ (x 10)	$O.D._f$ (x 10)	Color
0	6.4	0.75	208
1	6.1	0.74	200
2	6.0	0.74	185
4	5.8	0.72	172
8	5.7	0.73	170

All samples used in this study were analyzed within three days of collection and concentrated within one week.

In general, 25 gallons of each water was obtained. The source of each water and the designations used to describe them throughout this report are listed in Table 5.

Chemical Analyses

A partial mineral analysis was performed on each colored water. These data are presented in Tables 6 and 7.

It is evident that the majority of these colored waters were of low mineral content with the exception of the two waters from the Florida Everglades. Several other facts are evident from these analyses. First,

TABLE 5

Sources and Designations of Colored Waters

Water	Source
A	Creek near Newman's Lake, Gainesville, Florida
B	*Suwannee River at Fargo, Georgia
C	Rice Creek near Palatka, Florida
D	Lumber Creek near Raleigh, North Carolina
E	Juniper Creek near Raleigh, North Carolina
F	Florida Everglades about 20 miles NW of Miami
G	Drainage canal near Belle Glade, Florida
H	Emerson Brook near Tewksbury, Massachusetts
I	Great Dismal Swamp near Norfolk, Virginia
J	Unidentified stream near Hartford, Connecticut

*River rises in the Okefenokee Swamp and samples collected in the swamp near the source.

all of the colored waters contained an appreciable iron content. Attempts were made at the beginning of this work to obtain a water free of this metal. Eight Florida waters and one Georgia water, all from different sources and types of drainage areas, were secured in this endeavor and all were found to possess substantial iron contents as seen in Table 8. In addition, Nordell (76) lists the results shown in Table 9.

Second, there is no relation between iron content and color value for waters from different sources, although for a given water this might be expected with seasonal variations.

Third, the chemical oxygen-demand values do not vary linearly with color values in waters from different sources, although this relation might hold for a given water. Fourth, biochemical oxygen-demand values are extremely low in all cases. If the organic substances responsible for color in water are products of microbiological decomposition, they are apparently in their final state.

Finally, the amount of dissolved matter in a water sample may often be estimated by multiplying the specific conductance by an empirical factor, that varies from 0.55 to 0.90. The data in Tables 6 and 7 show that for soft waters containing organic color this factor is appreciably higher, varying from 1.47 to 2.21.

Effect of pH on Color

One of the most interesting chemical properties of natural colored water is the variation of color intensity with pH. This "indicator" action has been noted by many authors, but again little data on the extent of this effect has been presented in the literature.

Each colored water sample used in this study was diluted 1:1 with a Clark and Lub's buffer solution of desired pH value, and the resulting color determined on a Lumetron. These data for waters A, B, D, E and G are presented in Figures 1 through 3, and Table 10. In every water used in this study the variation of color with pH was reversible, but not linear. In most cases, the slope of the curve in acid solution was considerably less than in basic solution. The data in Table 10 show that the per cent increase in color value over the pH range employed was quite variable. However, the greatest increase was obtained in waters with

TABLE 6

Chemical Analyses of Waters A Through E

	A	B	C	D	E
Total hardness ppm as $CaCO_3$	9.6	14	68	40	30
Ca ppm as $CaCO_3$	---	8.0	40	2.5	2.0
Mg ppm as $CaCO_3$	---	6.0	28	1.5	1.0
Alkalinity ppm as $CaCO_3$	10	0.0	45	2.0	1.3
Chloride ppm	10	16	116	3.0	3.0
Total iron ppm	0.58	1.0	0.31	1.5	0.40
pH	6.75	4.26	7.28	5.85	5.50
Color	240	352	156	108	68
Turbidity ppm	trace	12	trace	trace	trace
Conductivity μmhos/cm at 25°C	44.0	42.5	256	22.0	17.0
Total residue at 180°C ppm	76	94	250	40	25
COD ppm from dichromate	142	83	46	96	28
BOD ppm 5 days at 20°C	1.24	---	0.99	0.60	1.6

TABLE 7

Chemical Analyses of Waters F Through J

	F	G	H	I	J
Total hardness ppm as $CaCO_3$	295	505	30	16	8.0
Ca ppm as $CaCO_3$	250	365	24	10	6.0
Mg ppm as $CaCO_3$	45	140	6.0	6.0	2.0
Alkalinity ppm as $CaCO_3$	230	370	10	4.0	2.0
Chloride ppm	70	183	5.0	4.0	2.0
Total iron ppm	0.15	0.30	0.12	1.05	0.70
pH	7.85	7.95	6.90	5.10	5.00
Color	76	264	70	424	240
Turbidity	trace	4.0	trace	10	4.0
Conductivity μmhos/cm at 25°C	770	1490	88	61	23
Total residue at 180°C ppm	469	990	65	113	61
COD ppm from dichromate	51	142	24	66	64
BOD ppm 5 days at 20°C	1.7	4.7	0.6	1.1	1.4

TABLE 8

Variation of Iron Content in Colored Water Samples

Water Source	Fe (ppm)	Color (ppm)
Suwanee River at Santa Fe	1.08	102
Suwanee River at Fargo, Ga.	0.78	316
Fort Pierce	0.50	182
Palm Beach Canal	2.40	88
U. S. 27-South Bay	1.39	65
Pumping Station-U. S. 27	0.36	76
Miami Canal at Bend	1.11	60
Tamiami Trail Canal	0.97	45
Newnan Lake tributary	0.58	220

TABLE 9

Variation of Iron Content in Colored Water Samples

Water Source	Fe (ppm)	Color (ppm)
Great Dismal Swamp Elizabeth City, N. C.	1.8	1200
Haw River Burlington, N. C.	1.2	300
Satilla River Waycross, Ga.	0.11	130
Charles River Shannuck, R. I.	0.20	70
Black water River Burdette, Va.	0.17	80
Rancocos Creek Birmingham, N. J.	2.00	100

TABLE 10

Variation of Color with pH

Water	Color at pH 2.0	Color at pH 10	Per Cent Increase Over Color at pH 2.0
A	160	270	67
B	237	412	74
C	102	182	78
D	71	130	83
E	50	82	64
F	54	82	52
G	190	290	53
H	42	86	105
I	330	440	30
J	150	265	77

lowest original color, and the smallest increase was obtained in waters with the highest original color.

Because of this pH-color variation, all color values reported in the course of this work were measured at a constant pH of 8.4.

Particle Size Estimation

Natural color in water has been reported as being in true solution by some authors (20) and as existing mainly as a colloidal dispersion by others (77). In all cases, the proposed theories have been backed by little or no experimental evidence on particle size.

Early in this study it was observed that the smallest commercially available membrane filter, a 10 mμ millipore filter, would retain only 13 per cent of the original color value of water A. Accordingly, two electrodialysis cells were built. They were constructed from plexiglass, fitted with a copper gauze cathode and a graphite block anode. These cells were essentially rectangular plastic boxes cut apart at approximately one-third and two-thirds of their total length for the insertion of membranes and held together with steel bolts through plastic cross-braces at either end. The only difference in the two cells was their capacities, namely one and three liters.

In operation the cell was wired directly to a 125 volt direct current line in the laboratory. A milliammeter was connected in series with the anode and a voltmeter was tapped across the electrodes. The electrode compartments were filled with distilled water and changed hourly.

The maximum amount of color that would dialyze through a fairly coarse parchment paper membrane was found to be 82 per cent of the original color after 16 hours of continuous operation. Unfortunately, the pore size of this coarse parchment could not be determined. However, when water A was dialyzed through a cellophane membrane with a pore size of 4.8 mμ, 87.5 per cent of the color was retained after 24 hours. Collodion membranes held 91 per cent of the color in the same length of time. These data are summarized in Table 11.

These data indicate that most of the color in this water is colloidal in nature, and while there is a distribution of particle sizes, most of the particles are between 3.5 and 10 mμ in average diameter.

TABLE 11

Electrodialysis and Millipore Filtration of Water A

Material	Pore Size	Maximum Per Cent Color Retained
Millipore filter	10 mμ	13.0
Cellophane membrane	4.8 mμ	87.5
Collodian membrane	3.5 mμ	91.0

All of the waters used in this study were vacuum filtered through millipore filters of graded pore size down to 10 mμ. In addition, each water was dialyzed for 24 hours using the 4.8 mμ cellophane membrane. The effects of each on the residual color values of the waters are presented in Figures 4 and 5.

It is apparent from these figures that a natural distribution in particle size existed in all of the colored waters used in this study. Whereas the per cent of original color retained by the 100 mμ filter varied from 4 to 38 per cent, that retained by the 4.8 mμ membrane varied only slightly and was approximately 90 per cent. In every colored water the majority of the particles were between 4.8 and 10 mμ in average diameter. Furthermore, approximately 10 per cent of the color of each water passed through a membrane with a pore size of 4.8 mμ.

The light-scattering properties of turbidity suspensions have been extensively investigated by Black and Hannah (78). These authors characterized various natural and synthetic dispersions by measuring the intensity of scattered light from 15° to 137° per unit intensity of transmitted light at 0°. In a given suspension, the ratio of scattered

intensity at 15° per unit scattered intensity at 90° per unit volume was defined as a scattering ratio. For Latex dispersions, the scattering ratio increased with increasing particle size as shown in Table 12.

TABLE 12

Scattering Ratios of Latex Suspensions

Particle Size	Scattering Ratio
365 mμ	136
264 mμ	25
188 mμ	10
88 mμ	8

The upper limit of this ratio for substances in true solution is around 2.0.

If organic color in water is particulate rather than dissolved, a scattering pattern should be obtained with a sensitive light scattering photometer. Accordingly, water B was filtered consecutively through a 100 mμ and a 10 mμ millipore filter, as this procedure was found to produce an optimum turbidity-free water by Black and Hannah. Scattering intensities of a sample prepared in this manner were measured from 15° to 137° with light sources of 436 and 546 mμ. Fluorescence filters were supplied with this instrument for each wavelength and were color filters that transmitted no source radiation. The results are shown in Figures 6 and 7, and describe the variation of scattering intensity at any angle per unit transmitted intensity at 0° with the angle θ. It is evident that

these materials show appreciable scattering properties at 546 mμ and that the scattering ratios are not those of dissolved materials.

Scattering at 436 mμ is more intense and relatively constant from 40° to 137°. This is caused by fluorescence of the organic color at this wavelength and is shown in Figure 11 to be very intense and independent of the angle θ. No fluorescence was observed at 546 mμ. It is interesting to note the effect of pH on the scattering properties of this water. As shown in Figure 6 raising the pH from 5.0 to 12.0 resulted in a decrease in the scattering ratio, whereas the scattered intensity at any angle per unit transmitted intensity at 0° was higher. These data indicate that raising the pH of this colored water effected both a decrease in individual particle size and an increas in the number of particles present. The fluorescence intensity was higher in alkaline solution as had been determined previously.

Coagulation Behavior

Black and Willems (77) have shown that coagulation of organic colored water proceeds by an electrokinetic mechanism. They state, "When alum or ferric sulfate is added to a colored . . . water whose alkalinity, naturally present or added, is sufficient to produce a pH value within the upper portion of the pH range of hydrolysis of the respective salts, . . . positively charged colloidal micelles . . . are formed. These positively charged hydrosols neutralize the negative charge on the colloidal particles of color . . . resulting in electrokinetic coagulation."

Although the two colored waters used in their study were of different total solids and alkalinity content, they were both local waters and were obtained from areas with similar forest covers.

As a knowledge of the similarity of this behavior in waters from widely different sources is desirable, jar test and electrophoretic mobility data were obtained on seven of the waters used in this study. Water A was not run as it was one of the waters used by Black and Willems and had not changed in quality since their determinations. Waters F and G were not coagulated as normal treatment of these waters would have been lime softening.

The remaining waters were coagulated with alum, adjusting the pH when necessary with lime or HCl. Figures 8 and 9 show the results for waters B and I.

The uniformity of coagulation behavior in these colored waters is apparent from these figures. The zone of optimum color removal was accompanied by a zone of charge reversal in every case.

In waters B, C, I, and J, both zones occurred within relatively narrow pH ranges. Waters D, E, and H had considerably wider pH ranges of optimum color removal. However, the alum dosages employed with these waters were substantially higher than would be used in normal plant practice for waters of such low color values.

It is interesting to note that the width of the pH range of charge reversal for these waters was roughly proportional to the width of the pH range of optimum color removal as is shown in Figure 10.

These data indicate that in this respect at least, organic colored waters from various sources are remarkably similar.

The fact that optimum coagulation of colored waters occurs at relatively low pH values has been ascribed entirely to the low pH ranges of hydrolysis of the Al (III) and Fe (III) ions (77). It should be mentioned here that light scattering data obtained in this study

have shown that the particle size of color particles is affected by pH. In acid solution the color particles were found to be fewer in number and larger in size than those in the same water in basic solution. What effect this phenomenon has on coagulation is undetermined but it should be considered if the exact mechanism of color coagulation is to be understood.

Fluorescence Spectra

Shapiro used the fluorescence of organic color fractions to locate bands after chromatographic separation on paper. However, the fluorescense emission spectra of organic color have never been recorded. Since it was observed in this work that the raw stream waters fluoresced as brilliantly as some of the fractions isolated from them, the spectra of both were determined. Only the raw water fluorescence spectra will be presented here.

Figure 11 is the spectrum of the Osram mercury lamp source with distilled water, while Figures 12 and 13 are the fluorescence spectra obtained from waters B and F. It can be seen from these figures that the emitted fluorescence has a maximum intensity at 490 mμ although the bands are relatively broad and of appreciable intensity from 450 to 550 mμ. The spectra of the remaining waters varied only in the intensities of emission.

The different mineral content of the waters may account for the fact that the relative intensities of fluorescence emission were not proportional to the initial color values.

Adjusting the pH value of any of the colored water samples to higher values would invariably increase the intensity of fluorescent emission. This effect is shown for water B in Figure 14.

Ultraviolet Spectra

None of the colored waters investigated in this study showed any absorption in the visible region and all showed only end absorption in the ultraviolet region. Each water was diluted, if possible, with pH 8.0 buffer to color values of 200, 100, 50, 30 and 10 color units as determined on the Lumetron. These samples were scanned on the Cary Model 14 from 200 to 600 mμ against the same concentration of pH 8.0 buffer. Two of these spectra are presented in Figures 15 and 16. However, all were similar in two respects. First, each water showed only end absorption and second, dilution of each water had no effect on the nature of the absorption curve other than to decrease the absorbancy value. A substantial variation was observed in the absorbancy values of each water at 200, 300 and 350 mμ as shown in Figures 17 through 19. However, at 350 mμ all of the waters with the exceptions of waters F and G had essentially the same absorbancy value at all concentrations of organic color. The two exceptional samples were both obtained from the Florida Everglades, in a region of extremely uniform vegetative cover, and were noticeably more yellow to the eye than the rest of the samples used in this study. In addition, waters F and G exhibited very weak fluorescence spectra, in comparison with the other colored waters.

The effect of pH on the ultraviolet spectra of waters B and C is shown in Figures 20 and 21. Only two waters are shown here, as identical results were obtained with every water. The absorbance value in alkaline solution was always uniformly higher than that in acid solution.

The facts that organic colored waters are invariably colored yellow-orange to the eye yet show no absorption in the 450-550 mμ

region, and that the general shape of the ultraviolet spectra is that of a scattering rather than an absorption curve suggests that the color in these waters is due to scattering rather than molecular absorption of light energy. The difference in absorbance values at the wavelengths shown in Figures 17 through 19 are probably due to two factors. First, a natural variation in particle size would account for a variation in scattering intensity. Second, the color values for each series were determined on a Lumetron filter photometer which detects scattered, transmitted and fluorescent radiation. The Cary, however, eliminates all light of different wavelength from the incident beam with a second monochromator, and therefore would not detect fluorescent radiation. Thus, the Cary spectrophotometer was not examining equal "color" values as determined on the Lumetron.

The variation of absorbancy on the Cary, as well as the Lumetron, with pH of the colored water samples may also be explained by changes in particle size and fluorescence intensity with pH. As mentioned in a previous section, both of these effects have been observed.

These data are consistent with the theory that color in water is of a colloidal nature.

Characteristics of Extracted Organic Material

Nature of the Fractions

Any fractionation scheme that is to be used in a method of classification of colored waters should include all of the organic matter present in these waters. The only fractionation method reported in the literature has been presented by Shapiro (22). However, this author

reported that some organic matter was excluded by this extraction procedure, which was intended to extract one relatively pure acid fraction. To determine if this amount was significant, water B was concentrated and fractionated using Shapiro's techniques.

Shapiro's fractionation scheme was essentially as follows: The concentrate obtained by vacuum distillation was dried in an oven at 60°C. The solid thus obtained was mixed with 80 per cent ethanol acidified with HCl. This mixture was taken to dryness, dissolved in dilute HCl and extracted with various organic solvents, the most efficient of which was ethyl acetate. Shapiro referred to material obtained in this manner as the "free acid" fraction. Usual concentrations of this fraction were reported as 4.16-5.24 mg/l of raw water.

The solid and liquid materials resulting from the concentration of 25 gallons of water B were combined and evaporated to dryness in an oven at 45°C over a 48-hour period. This solid residue was treated with 80 per cent ethanol acidified with HCl to remove mineral materials. The major portion of the residue dissolved leaving a small but definite amount of finely dispersed solid (I). The alcohol solution was then evaporated at 45°C leaving a dark colored solid which was mixed with dilute HCl and extracted with ethyl acetate thereby separating it into two more fractions; (II)', acid soluble but insoluble in ethyl acetate and (II) soluble in ethyl acetate. Fraction (II)' can be further separated yielding a water-soluble portion (III) and a water-insoluble fraction (IV).

Thus four distinct fractions were obtained on the basis of solubility by Shapiro's extraction scheme.

Tables 13 and 14 summarize the solubilities and relative distribution of material in these fractions.

TABLE 13

Solubility Relationships of Fractions Obtained by Shapiro Technique

Fraction	H_2O	NaOH 5 per cent	$NaHCO_3$ 5 per cent	EtOH 95 per cent	Ethyl Acetate
I	-	+	+	-	-
II	±	+	+	+	+
III	+	+	+	+	-
IV	-	-	-	+	-

+ soluble - insoluble ± partially soluble

TABLE 14

Weight Distribution in Fractions Obtained by Shapiro Technique

Fraction		Weight Per Cent	Weight (from 25 gal)
	I	7	0.36 gms
"free acid"	II	26	1.34 gms
	III	8	0.41 gms
	IV	59	3.06 gms

Thus, the free acid fraction described by Shapiro is seen to contain less than 30 per cent of the organic material present in this natural water.

All of these fractions dried to a brown to black solid, I and III being noticeably lighter in color than II and IV. Fractions I, III, and IV gave no melting points, i.e., they were stable up to 360°C. However, fraction II softened at 90-100°C. This is a somewhat narrower melting point range than that reported by Shapiro, but still indicates a mixture of several constituents.

All of these materials were insoluble in non-polar organic solvents such as benzene, petroleum ether and dioxane. I, II, and III were only slightly acid soluble but were completely soluble in basic solutions of 5 per cent NaOH and 5 per cent $NaHCO_3$. Fraction IV was only partly soluble in alkalis but was very soluble in dilute HCl.

The presence of enolic structures in all four fractions was tested by reacting these materials with bromine water and potassium iodide. All fractions gave a strongly positive test taking up bromine immediately and yielding a blue color with starch.

A positive test for aromaticity was obtained on all fractions with the Chloranil test. However, the Le Rosen test for aromaticity was negative for each fraction. Comparative tests with pyrogallol and anthraquinone gave the same results. In the presence of sodium carbonate, fraction II gave an immediate red color with the diazonium salt of sulfanilic acid, indicating a phenolic nature.

Aqueous titration of fraction II with NaOH yielded an equivalent weight of 224 gms/eq. Shapiro reported an equivalent weight of 228 gms/eq for the "free acid" fraction isolated from a Connecticut colored water.

The infra-red absorption spectrum of fraction II, the equivalent of Shapiro's free acid fraction, is shown in Figure 22. This spectrum was identical with one obtained in Shapiro's laboratory.

In general, the data obtained in this study on fraction II are consistent with those reported by Shapiro for the free acid fraction.

As mentioned earlier in this report, many authors have pointed out the vast variety of humus substances of varying elemental compositions that may be obtained from soil organic matter, the particular compositions depending on the type of soil, manner of extractions and strength of extractants. Since it is believed that these materials do originate in the soil and in bottom deposits as well, it was decided to try a manner of separation more similar to that outlined by Oden, Waksman and Bremner which has since become the classical method of fractionation of soil organic matter.

Whereas less than 30 per cent of the total organic matter present was isolated by Shapiro's scheme, the classical separation of humic material in water would, if applicable, separate the entire organic content into generally recognized fractions of humus material as shown in Figure 23.

The concentrate used in this part of the study was prepared by both concentrating techniques so that a comparison could be made.

Many soils contain ether or chloroform-soluble fats and waxes, the actual type and amount depending on the nature of the humus and its abundance in the soil. Accordingly, one concentrate batch obtained by freezing was extracted in the following manner. One hundred milliliters of concentrate was shaken in a separatory funnel with 200 ml of sodium-dried ether. The ether was drawn off, dried over Na_2SO_4 and allowed to evaporate. The residue consisted of a greenish-yellow, odorless wax. Two extractions of ether were sufficient to remove all the wax from this volume of concentrate. Only 2.0 mg was obtained from 100 ml of concentrate.

The wax melted at 55-60°C and gave no indication of decomposition up to 130°C. Attempts were made to identify this material in methanol in a vapor phase chromatography apparatus. However, nothing was recorded but solvent up to a retention time of 10 minutes.

Solid material was noted in the concentrate obtained by either the freezing or evaporation methods although there seemed to be less in freeze concentrates. This solid was at first included in the extraction process since the entire concentrate was taken to dryness before extraction.

Since the first step of the new fractionation scheme required a precipitation of the liquid concentrate with HCl, it was decided to remove this solid. A 0.45 μ millipore filter was sufficient for this purpose and 3.5 gms of solid was filtered from one concentrate batch before the HCl treatment.

When the filtrate was treated with HCl, a much finer solid was formed. These solids, namely that produced in the concentration process and that produced by HCl treatment, were labeled fractions Ia and Ib respectively.

Samples of each were subjected to an elemental analysis. The results, on an ash free basis, are shown in Table 15.

Throughout the literature search for this work, all references to the separation of the combined humic and hymatomelanic acids by the addition of HCl referred to the separation as a precipitation process. The general appearance of both of these solids, and the manner in which they were formed suggested that they were not true precipitates but were formed from a colloidal fraction in the concentrate and were actually flocculated particles. A solid obtained by adding HCl to an

extract of soil obtained from the stream area could not be distinguished from one obtained from the water.

TABLE 15

Ultimate Analyses of Fractions Ia and Ib

	Per Cent C	Per Cent H	Per Cent N
Ia	35.50	5.40	1.86
Ib	43.90	4.75	1.96

Fraction Ia probably resulted from a compression of the electrical double layer during concentration of the negative charged colloidal particles in the water to a point where its stability was overcome.

In order to avoid these physical or chemical changes during concentration, attempts were made to concentrate smaller original volumes which would still yield sufficient organic material for subsequent analyses. The concentration of 20 liters of waters having a color of 200 or more to 1.0 liter produced no settleable solid phase. For waters having a color of less than 200, a 40 to 1.0 concentration ratio was used with the same result.

All of the colored waters used in this study were concentrated in this manner. The weight of organic material in each fraction obtained from either 20 or 40 liters of raw water is listed in Table 16, while the weight percentage of each fraction in the raw waters is shown in Table 17.

TABLE 16

Weight of Organic Matter in Fractions

Water	Volume Concentrated (l)	Weight in Fraction, grams I	II	III
A	20	1.01	0.020	0.130
B	20	1.58	0.014	0.210
C	20	0.850	0.020	0.100
D	40	0.736	0.007	0.079
E	40	0.560	0.002	0.070
F	20	0.280	0.003	0.036
G	20	1.190	0.010	0.200
H	40	0.499	0.013	0.047
I	20	2.07	0.020	0.310
J	20	1.02	0.008	0.205

TABLE 17

Percentage of Total Organic Matter in Fractions

Water	Fraction Per Cent		
	Fulvic Acid	Hymatomelanic Acid	Humic Acid
A	87.0	11.2	1.8
B	87.5	11.6	0.9
C	87.7	10.3	2.0
D	89.6	9.6	0.8
E	88.8	11.1	0.1
F	87.9	11.3	0.8
G	85.0	14.3	0.7
H	89.5	8.4	2.1
I	86.4	12.9	0.7
J	82.8	16.6	0.6

These data show that organic materials in colored waters are predominately of the fulvic acid type, and that the relative amounts in each fraction were fairly constant in all the waters examined.

A general relationship was observed between the total organic matter in each water and the color value of the water. As is shown in Figure 24, a straight line results if the total organic content, expressed here as mg/l of raw water, is plotted against the respective color values of the waters. Furthermore, samples of humic and hymatomelanic acids obtained from water A were found to follow Beer's law as shown in Figure 25. It is apparent from these data that on a unit weight basis, the fulvic acid fraction is the most color producing, but only slightly more so than the hymatomelanic acid fraction. Humic acid produces measurably less color per unit weight than either of the other two fractions.

Because of the predominance of the fulvic acid fraction in colored waters, most of the attention during this investigation was directed toward it.

Evidence of Functional Groups and Structural Characteristics

The fulvic acid material dried to a brown solid which was slightly hygroscopic and possessed a faint smell of caramel. The hymatomelanic and humic acid residues, however, possessed no noticeable odor, were non-hygroscopic and were of different color; the former drying to a light brown, flaky solid, and the latter drying to a dense, shiny black solid. All were stable in the solid form at temperatures up to 360°C, although the fulvic and hymatomelanic acid materials darkened noticeably at 270°C.

All fractions gave colored mixtures in 5 per cent NaOH and 5 per cent $NaHCO_3$ leaving no solid material in the bottom of a test tube. Fractions I and III were partially dissolved or dispersed in EtOAc, and completely so in ethylenediamine, pyridine and dimethylformamide. All of these materials were insoluble in non-polar organic solvents such as benzene, petroleum ether and hexane.

All enols take up bromine instantly with intermediate formations of dibromo-enols which form labile α-bromoketones on elimination of HBr. Such α-bromoketones oxidize hydroiodic acid and liberate free iodine. Fulvic acid samples from every water used in this study gave a positive reaction to this test yielding a blue color with starch. In most cases, the reaction and subsequent color development was much slower than the model compounds aceto-acetic and benzoylacetic esters.

The same organic spot tests used with the Shapiro fractions were employed to determine the presence of aromaticity in the fulvic acid fractions of each water. The chloranil test was positive for the fulvic acid fraction of every water except waters C, F and G, while the Le Rosen test with sulfuric acid and formaldehyde was negative in all cases. However, the strongest evidence of aromaticity in this fraction was obtained using the phosphotungstic-phospho-molybdic acid reduction test. Aromatic hydroxyl compounds reduce these acids with the production of a characteristic blue color. A strongly positive reaction was observed for every fulvic acid sample obtained in this study.

Infra-red spectra of the fulvic acid fraction of each colored water were obtained and were very similar as shown in Figures 26 and 27 and Table 18.

Carboxylic acids typically show broad absorption bands with a series of minor peaks or satellite bands over the range of 3.33 to 4.0μ.

TABLE 18

Infra-Red Absorption Bands of Fulvic Acid Fractions

Water	Band location, μ					
A	3.30-4.00	5.85	6.15	6.95	7.20	8.00-10.0
B	3.30-4.00	5.82	6.18	6.95	7.25	7.80-10.0
C	3.28-4.15	5.80	6.18	6.95	7.25	7.80-10.2
D	3.31-4.00	5.85	6.15	6.97	7.20	7.95-10.1
E	3.31-4.00	5.85	6.15	6.97	7.15	7.90-10.1
F	3.32-4.00	5.85	6.20	6.90	7.20	7.95-10.0
G	3.35-3.98	5.85	6.15	6.90	7.20	7.95-10.0
H	3.30-4.15	5.85	6.15	6.95	7.20	7.80-10.0
I	3.28-4.20	5.85	6.15	6.95	7.20	8.10-10.0
J	3.30-4.15	5.85	6.15	6.95	7.20	8.30-10.0

This is a result of the relative proximity of the associated OH and normal C-H stretching frequencies. These bands were observed on the spectra of all fulvic acid samples.

The two strongest absorption bands on every spectrum were located at 5.8 and 6.15μ . The former is the carboxyl group stretching frequency while the latter is the characteristic stretching frequency of doubly bound carbon atoms in conjugated phenyl groups.

Absorption in the range of 6.9 and 7.3μ in all samples was probably due to alkane groups. The C-H deformation frequencies of both the $-CH_2-$ and CH_3-C- groups are in this region as are the C-H stretching frequencies of the cis and trans isomers of $R_1CH=CHR_2$.

The very broad absorption regions between 8.0 and 10.0μ is probably due to a combination of the C-C and C-O stretching frequencies. The fact that this region is very broad and lacks any more definite absorption bands indicates that these materials are structurally complex and/or contain several carboxylic acids.

Figure 28 is the infra-red spectrum of the methyl ester of the fulvic acid from water A. Diazomethane was used as the methylating agent. The presence of the band at 2.9μ indicates that not all of the aromatic hydroxyl groups were methylated. The increased absorption at 3.4μ must be attributed to the increase in methyl groups. The absorption at 6.9μ is stronger than in the unmethylated fulvic acid while that at 7.3μ is weaker. In addition several bands were obtained, namely at 7.95, 9.15, 9.80 and 12.5μ respectively. The band at 12.5μ may well be the only spectral information obtained to date on the nature of the substitution of the aromatic nuclei in these molecules. The C-H

out-of-plane deformation frequencies of the three adjacent free hydrogen atoms in 1,2,3-trisubstituted benzene characteristically occurs at 12.50-12.99μ .

The infra-red data thus confirm the assumption that these materials are hydroxy carboxylic acids, and give strong indications of unsaturation and aromaticity. The negative charge on particles of organic color may arise from ionization of the carboxyl and aromatic hydroxyl groups.

Fifty milligram samples of the three fractions of water A were sent to Galbraith Laboratories, Inc., for ultimate analysis. The results, on an ash free basis, are shown in Table 19.

TABLE 19

Ultimate Analyses of Fractions of Water A

Fraction	Per Cent C	H	N	O
I	41.50	5.72	1.98	50.80
II	29.30	5.94	1.85	62.91
III	49.29	5.11	1.24	44.36

A similar analysis of the fulvic acid fraction of water B showed 40.6 per cent C, 5.45 per cent H, 1.53 per cent N and 49.78 per cent O. The fulvic acids of these two waters are indeed very similar in this respect. In addition, the fulvic acid fraction of water B showed a methoxyl content of 2.64 per cent.

Complexity of Fractions

Some knowledge of the complexity of these fractions was obtained by the use of paper chromatography. Several solvent systems were tried but the most useful had the following composition by volume: pyridine, 50 ml; water, 50 ml; 2-methyl-2-butanol, 50 ml; $(C_2H_5)_2NH$, 2 ml.

An excellent elution solvent was composed of methyl ethyl ketone, formic acid and water of the following volume composition: methyl ethyl ketone, 37.5 ml; water, 15 ml; formic acid, 7.5 ml.

The following tables summarize the results obtained by chromatographing the three fractions of water A on Whatman paper number 3 by the descending technique. The bands were located by irradiation with ultraviolet light.

TABLE 20

Paper Chromatographic Separation of Fulvic Acid Fraction of Water A

Band No.	Rf Range	Description
1	0.0	dark brown (origin)
2	0.10-0.16	dark yellow (major)
3	0.36-0.38	yellow (major)
4	0.39-0.63	yellow smear
5	0.67-0.80	yellow smear

TABLE 21

Paper Chromatographic Separation of Humic Acid Fraction of Water A

Band No.	Rf Range	Description
1	0.0	brown (origin)
2	0.40-0.50	yellow (major)
3	0.44-0.61	yellow
4	0.93-0.98	yellow

TABLE 22

Paper Chromatographic Separation of Hymatomelanic Acid Fraction of Water A

Band No.	Rf Range	Description
1	0.0	brown (origin)
2	0.08-0.11	yellow

Several of the bands obtained by chromatographing the fulvic acid fraction of water A were eluted for infra-red analysis. No significant difference in the absorption spectra of any band from that of the unseparated fulvic acid was noted.

Titration in Non-Aqueous Solvents

The acid strength of a phenolic hydroxyl group is influenced by the nature and position of any other substituents which may be present.

Phenol itself, and the naphthols and their homologues are only weakly acidic in aqueous solution. Similarly, the fulvic acids isolated from colored waters in this study, which have been shown to contain both carboxylic acid groups and phenolic hydroxyl groups, exhibited only a slightly acidic nature in aqueous solution.

In order to increase the acidity of these groups and obtain more well-defined equivalence points, it was necessary to employ basic organic solvents. Dimethylformamide, butylamine and ethylenediamine were tried but the most useful was ethylenediamine. As the solutions of fulvic acid in this solvent were intensely colored, it was necessary to titrate them potentiometrically. A platinum-saturated calomel electrode combination was found to be very suitable when sodium aminoethoxide was used as the titrant. Standardization of the sodium aminoethoxide was accomplished with benzoic acid. A typical potentiometric titration curve of this acid is shown in Figure 29.

All the solvents were redistilled before use, as even traces of water were found to significantly decrease the magnitude of the potential change at the equivalence point.

Several model compounds were titrated to evaluate the magnitude of this break for the neutralization of carboxylic and phenolic acid groups. Figure 30 shows the curve obtained by titrating 53 mg of phenol with 0.148 N sodium aminoethoxide. A sharp break of 125 millivolts was obtained and calculation revealed an experimental equivalent weight of 94.0 compared with the theoretical value of 94.11. Two gradual breaks were observed for resorcinol as shown in Figure 31.

This procedure will distinguish between carboxyl and phenolic acidity in the same molecule as shown by the ortho and parahydroxybenzoic

acid curves in Figures 32 and 33. These data show that the o-hydroxy acid is the stronger acid of the two, but that the phenolic hydrogen on the p-hydroxy acid is more acidic than that on the o-hydroxy acid. This difference in acidity may be attributed to hydrogen bonding in and subsequent stabilization of the salicylate anion.

The fulvic acid fractions of waters A through J were titrated in this manner and the calculated equivalent weights are shown in Table 23. The shapes of the titration curves were very similar and each contained one well-defined equivalence point, as shown in Figures 34 and 35, for the fulvic acid fractions of waters B and I. These data indicate that if different types of acid groups exist in these materials, they are essentially of the same acidity. The other titration curves were similar in appearance although the equivalent weights calculated from them varied significantly. This apparent natural difference in the number of acid groups per mole was also shown from quantitative methylation data.

Methylation of several model compounds using the procedure described in the chapter on Experimental Procedures gave the results listed in Table 24. The values listed are the maxima that could be obtained with this methylating agent. It is apparent from these data that diazomethane will not completely methylate all of the phenolic hydroxyl groups in these compounds. If the structural relationships in the fulvic acid molecules are at all similar to the structures of these model compounds, the validity of the assumptions listed above is doubtful. Nevertheless, the former definition of the $\bar{n}$ value is valid and should be an approximation of the number of acid groups in these fulvic acid fractions per unit weight. Table 23 lists the $\bar{n}$ values

TABLE 23

Equivalent Weight and Quantitative Methylation Data of Fulvic Acid Fractions

Water	Equivalent Weight gms/eq	$\bar{n}$
A	108	1.34
B	106	1.35
C	89	2.02
D	111	0.48
E	114	1.18
F	138	0.20
G	135	0.41
H	133	0.45
I	122	1.12
J	105	1.40

TABLE 24

Quantitative Methylation of Model Compounds

Model Compound	Moles CH_2N_2 Reacted / Mole Sample	
	Theoretical	Experimental
hydrocinnamic acid	1.00	1.01
p-hydroxybenzoic acid	2.00	2.05
o-hydroxybenzoic acid	2.00	1.80
hydroquinone	2.00	1.55
resorcinol	2.00	1.62

obtained for the fulvic acid fraction of waters A through J, along with the equivalent weights of each as determined by titration in ethylenediamine.

It is interesting to note that the lowest equivalent weight was obtained for the fulvic acid fraction of water C, and that this same fraction showed the largest $\bar{n}$ value. In general, there was an inverse relationship between the equivalent weight and $\bar{n}$ values, although this relationship was not linear. The fact that the $\bar{n}$ values are not exactly 1.00 per unit equivalent weight indicates, for the $\bar{n}$ values less than 1.0, that some titratable groups are not methylatable, and for $\bar{n}$ values greater than one, some methylatable groups are not titratable.

Effects of Gaseous Oxidants

The most common method of color removal consists of coagulation with alum or ferric salts. However, several gaseous oxidants have been employed in plant practice to reduce organic color in water. The most extensively used of these is chlorine.

For over fifteen years, the city of Miami, Florida, has been treating a hard, colored water by lime softening followed by heavy chlorination. The softening process itself reduces the color value of the water from approximately 80 to a color value of 25 to 30. A chlorine dosage of 12 ppm reduces the remaining color to less than 10. Oxidation with ozone was tried on a pilot plant scale for a period of six months with excellent results. However, the power cost for the production of the ozone was found to be prohibitive. Although ozone is rarely used for water treatment in this country, over 200 municipal plants throughout Europe employ ozone for water disinfection, and for the control of tastes and odors.

Chlorine dioxide is used extensively in water treatment for taste and odor control, and in the paper manufacturing industry for the bleaching of wood pulp. This chemical, although more effective than chlorine as a disinfectant at high pH values, is generally too expensive for routine water treatment problems.

The effects of chlorine, chlorine dioxide and ozone on the color values of the waters used in this study were evaluated.

The gases were bubbled through duplicate 200 ml samples of each colored water concentrate until a constant value was obtained. The chemical oxygen demand of one sample was then determined according to the

procedures listed in Standard Methods, and the residual color value was measured on the other sample after it has been appropriately diluted. It was found that color values determined in this manner agreed with those resulting from oxidation of the raw water directly. In the cases of oxidation with chlorine and chlorine dioxide, it was necessary to destroy excesses of these chemicals with crystals of sodium thiosulfate before the color values could be determined on the Lumetron. The results are listed in Table 25.

It is apparent from these data that ozone was more effective than chlorine dioxide or chlorine for removing organic color, and chlorine dioxide was more effective than chlorine. Both chlorine and chlorine dioxide produced their maximum effects within a contact time of 5 to 10 minutes, whereas ozone generally required anywhere from 20 minutes to 1.0 hour. This is probably due to the relatively low concentrations of ozone employed.

In actual practice where these oxidants have been used for color removal, it has always been assumed that color removal corresponded to oxidation of the organic matter to carbon dioxide and water. That this is not true is shown by the results listed in Table 26. These data show that even in the absence of a color value the waters still exerted a substantial dichromate chemical oxygen demand. In addition, ether extraction of each concentrate sample that had been oxidized with chlorine yielded approximately 10 to 30 mg of a yellow solid which gave the infrared spectra shown in Figure 36 and Table 27. These spectra are very similar and much more definitive than those of the fulvic acid samples. Sodium fusions of each solid showed that no C-Cl bonds were present.

TABLE 25

Effects of Cl_2, ClO_2 and O_3 on Color Removal

Water	Original Color	Color after Oxidation Cl_2	ClO_2	O_3
A	240	--	8	0
B	352	98	14	0
C	156	72	10	0
D	108	30	3	0
E	68	25	0	0
H	70	15	15	3
I	424	22	18	3
J	240	5	0	0

TABLE 26

Effects of Cl_2, ClO_2 and O_3 on Chemical Oxygen Demand of Colored Water Concentrates

Water	COD of Concentrate	COD after Oxidation, ppm Cl_2	ClO_2	O_3
A	2800	--	750	615
B	1394	1060	80	220
C	996	118	40	152
D	1316	408	120	129
E	554	0	0	82
H	960	780	751	794
I	1320	830	652	742
J	1280	616	514	810

TABLE 27

Infra-Red Absorption Bands of Ether Soluble Chlorine Oxidation Products

Water	Band Location, Microns (μ)										
A	3.10	3.40	5.80	6.15	6.90	7.25	7.85	8.95	9.35	----	13.50
B	3.10	3.40	5.80	6.20	6.95	7.20	7.95	8.00-8.30	9.20-9.50	12.00-12.50	---
C	3.10	3.40	5.80	6.20	6.95	7.20	8.00	----	9.20-9.40	12.50	---
D	3.10	3.40	5.85	6.20	6.95	7.20	8.00-8.30	----	9.20-9.40	11.90-12.30	---
F	----	3.40	5.80	6.20	6.90	7.25	8.30-8.50	----	9.30-9.60	11.90-12.20	---
I	3.10	3.40	5.80	6.20	6.95	7.30	8.00-8.50	----	9.30-9.60	11.90-12.20	---
J	3.10	3.40	5.80	6.20	6.90	7.25	8.00-8.50	----	9.30-9.50	11.90-12.30	---

To determine if similar materials were present in a municipal water that had been treated with chlorine for color removal, 25 gallons of Miami tap water was concentrated to a final volume of 1.0 liter and extracted with ether. The extraction yeilded 65 mg of a solid which was similar in appearance to those previously obtained, and gave the infra-red spectrum shown in Figure 37.

The infra-red region from 11.1 to 16.7 μ is a region of strong, aryl, out-of-plane C-H deformation vibration absorptions which are valuable for identifying substitution patterns in aromatic ring systems. Generally, monosubstituted ring systems show absorption at 13.0 to 13.7 μ and 14.1 to 14.5 μ, o-disubstituted at 13.0 to 13.6 μ, m-disubstituted at 12.4 to 13.3 μ and 11.1 to 11.6 μ and p-disubstituted at 11.6 to 12.5 μ. The data in Table 27 show that with the exception of water A, all spectra gave bands that are characteristic of p-disubstitution. Since these materials have given every indication of being structurally complex, it must be concluded that this p-disubstituted ring is only a fraction of the whole structure and is perhaps an end group. All of these solids darkened at 285-310°C without melting and gave positive spot tests for aromatic hydroxyl groups.

Degradative Oxidation Studies

It is noteworthy that despite the many extensive investigations concerned with the structural nature of soil organic matter, no pure organic compound has ever been isolated that has been proven to exist in the natural organic complex. In addition, the small number of pure compounds that have been isolated have always represented yields of 10 per cent or less.

Similarly, the organic materials extracted from the colored waters used in this study were of such complexity as to defy identification by normal chemical procedures. It was observed that oxidation of fulvic acid with $KMnO_4$, $Ce(SO_4)_2$, ClO_2 and H_2O_2 produced ether-soluble oxidation products which gave infra-red spectra similar to the unoxidized fulvic acids. In all cases, mild oxidation resulted in a loss of fluorescence. However, some useful information was obtained by degrading these molecules by peroxy-acid oxidation. These studies were performed on the fulvic acid concentrates of waters A, C, I and J as these were the most highly colored.

Periodic acid has a selective oxidizing action on 1:2-glycols and on α-hydroxy aldehydes and ketones. Compounds of this type are cleaved at the C-C bond between the adjacent hydroxyl or carbonyl groups with the formation of aldehydes or acids of shorter chain length.

The procedure used for the oxidation of the fulvic acid concentrates with periodic acid was as follows. Two hundred milliliters of colored water concentrate was adjusted to pH 1.0 with dilute sulfuric acid and filtered to remove fractions II and III. This volume was then mixed with a solution of 1.0 gm of sodium paraperiodate in 50 ml of approximately 7 N H_2SO_4 in a 500 ml round bottom flask equipped with a reflux condenser. The solution was then refluxed at atmospheric pressure for 1.0 hour, cooled and extracted with ether.

In general, the extraction with ether left an aqueous phase that was only slightly colored whereas the ether solution was invariably a deep, orange-red color. Evaporation of the aqueous phases to dryness at 40°C under nitrogen and extraction with EtOH yielded solids which

gave infra-red spectra typical of the unoxidized fulvic acids. These solids represented approximately 10 to 20 per cent of the total weight of organic matter present after oxidation.

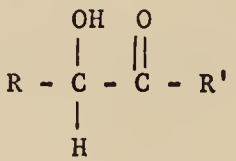
Chromatographic separation of the materials in the ether extracts was possible on a 10-inch column packed with adsorptive alumina. In general, the extracts gave one rather diffuse yellow band near the top of the column and one relatively narrow yellow band approximately 4 to 6 inches down the column. Neither of these bands fluoresced under ultraviolet light.

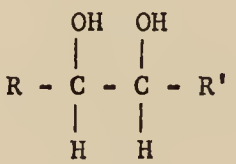
Following elution with 95 per cent EtOH, the lower band dried to a sweet smelling yellow oil while the upper diffuse band dried to an orange solid that decomposed in a Bunsen flame.

For water A, less than 15 mg of the oil was obtained, whereas 135 mg of material was obtained from the diffuse layer. The infra-red spectra of these materials resulting from the oxidation of water A are shown in Figures 38 and 39. The absorption data obtained with the yellow oil indicate that it is not a carboxylic acid and does not contain hydroxyl groups. The only absorption below 4.0 μ is due to the presence of C-H. The shoulder at 3.25 μ may be due to aromatic hydrogen while the strong peaks at 3.4 and 3.48 are the assymetric and symmetric C-H stretching frequencies of the CH_3-C group. The presence of the strong carbonyl band at 5.80 μ indicates that fulvic acids may contain these groups outside of carboxylic acid configurations. The doublet at 6.30 and 6.40 μ is probably phenyl ring absorption. This doublet frequently occurs in conjugated ring systems. The medium intensity bands at 6.90 to 7.30 μ are probably due to C-H deformations of the -CH_2- and CH_3-C groups respectively. Absorption at 7.90 μ is a result of C-C

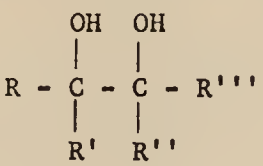
deformations while that at 8.95μ is due to C-O stretches. The origins of the bands at 9.40, 9.65 and 10.5 are uncertain while those at 13.5 and 14.25 are indicative of monosubstitution. Absorption in the 3.0-4.0 μ region in Figure 39 indicates that the solid materials obtained from this oxidation are a mixture of carboxylic acids. The band assignments for all bands up to 8.0 are the same as for Figure 38. Absorption at 9.3 and 9.60 μ is probably due to -OH deformations. The large band at 12.5 μ may be a result of skeletal vibrations. Attempts at further separation of these acids met with little success.

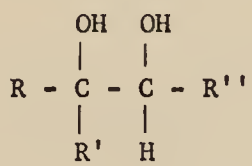
The data in Figures 38 and 39 suggest that fulvic acids contain either α,β-dihydroxy, or an α-hydroxy ketone groupings of the type,



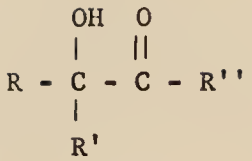


where both R and R' contain aromatic nuclei. The facts that the oily substance showed the presence of a carbonyl group but was not an acid, and that such small quantities of the oil were obtained, suggest that the following groupings may also be present in fulvic acids, namely





or



where R and R' contain monosubstituted phenyl rings.

V. SUMMARY

The physical and chemical properties of ten naturally colored waters have been investigated and have been found to be relatively independent of the source of the water.

Angular light scattering data and ultraviolet spectroscopy have shown that the materials responsible for color in water exist primarily in colloidal suspension in the water. It is suggested that the color in these waters is due to light scattering rather than molecular absorption of light energy. The pH value of the waters was found to affect both the particle size and number of particles in a given suspension. This effect was proposed as an explanation for the variation of color value with pH.

The particle size of the organic matter in each water was estimated at constant pH by dialysis and millipore filtration and was found to be generally less than 10 mμ.

The coagulation behaviors of these waters with aluminum sulfate were compared and it was observed that the pH zone of optimum color removal was accompanied by a zone of minimum zeta-potential and typically, of charge reversal, of the coagulated color particles.

The fluorescence spectra of these colored waters were recorded and revealed in all cases a maximum energy of emission at 490 mμ, with the combined excitation wavelengths of 365, 404 and 436 mμ.

Organic materials were isolated from these waters and were shown to be similar to natural soil organic matter. The predominate fraction

was found to be fulvic acid whereas the combined humic and hymatomelanic acid fractions accounted for less than 10 per cent of the total organic matter present in each water. For all the waters examined a linear relationship was observed between the amount of organic matter and the color value of the water.

Data obtained from solubility relationships, chemical spot tests and infra-red absorption spectra indicated that fulvic acids are aromatic polyhydroxy methoxy carboxylic acids. Ionization of the carboxyl and aromatic hydroxyl groups was offered as an explanation of the negative charge of color particles.

Equivalent weights of the fulvic acids were determined by potentiometric titration in ethylenediamine and were found to vary from 89 to 133 gms per equivalent among the waters studied. Quantitative methylations of the fulvic acids with diazomethane revealed that there were generally 0.40 methylatable groups present per 100 gms for fulvic acids of high equivalent weight and 1.35 methylatable groups per 100 gms for fulvic acids of low equivalent weight.

Ozone was found to be more effective than either chlorine or chlorine dioxide for the removal of color from these waters. It was shown that color removal did not correspond to oxidation of the organic matter to carbon dioxide and water. Chlorine oxidation was found to produce ether-soluble oxidation products. Infra-red spectra of the substances suggested that they are polyhydroxy carboxylic acids containing disubstituted phenyl rings. Similar materials were extracted from a municipal water system that routinely uses chlorine for color removal.

Degradative oxidation of the fulvic acids produced ether-soluble mixtures of complex carboxylic acids from which pure compounds could not be isolated. However, infra-red spectra of ether-soluble oxidation products of the fulvic acids with paraperiodic acid indicated that these acids contain α, β, dihydroxy, or α-hydroxy ketone configurations.

APPENDIX

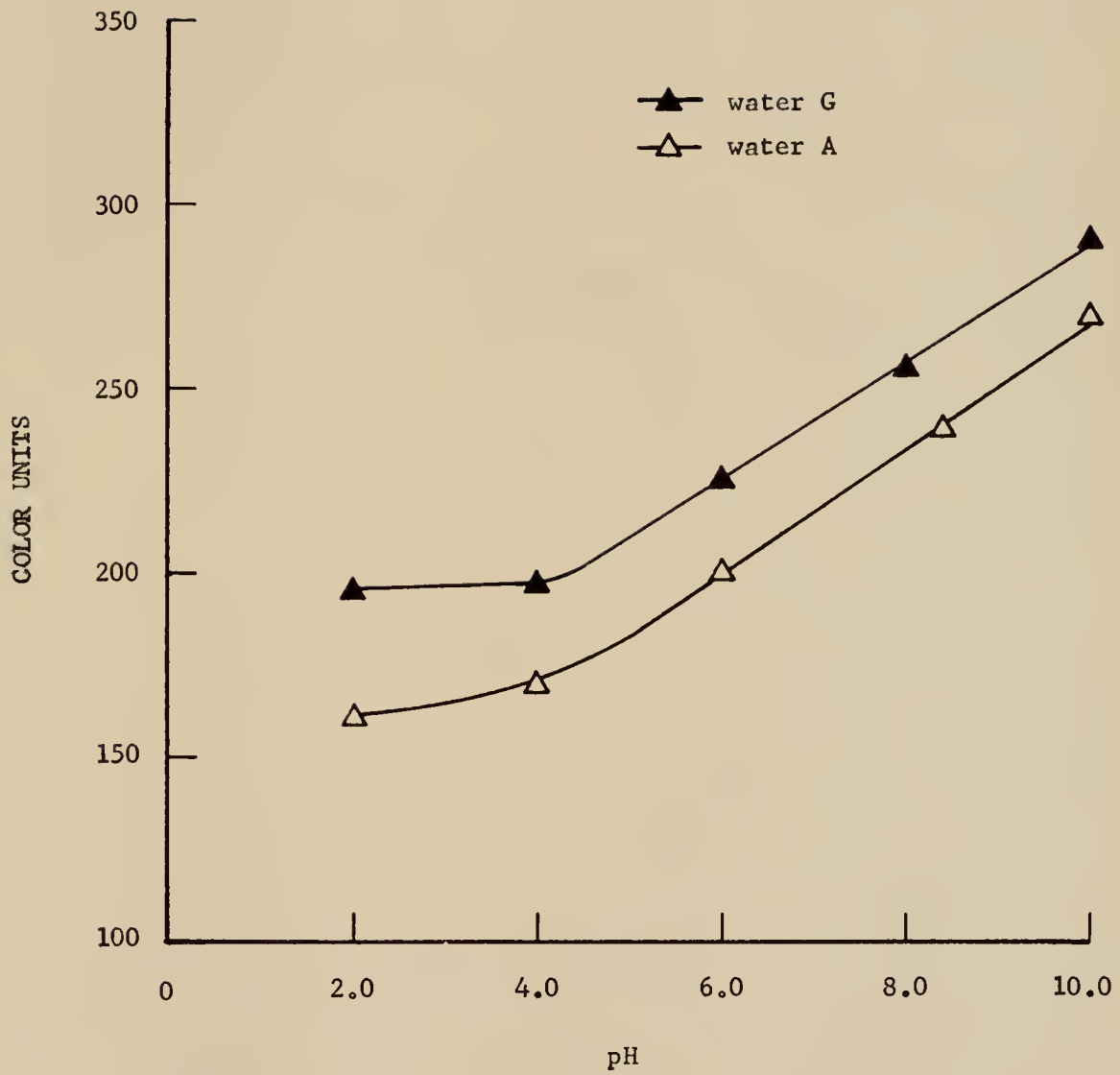


Figure 1. Effect of pH on Color of Waters A and G

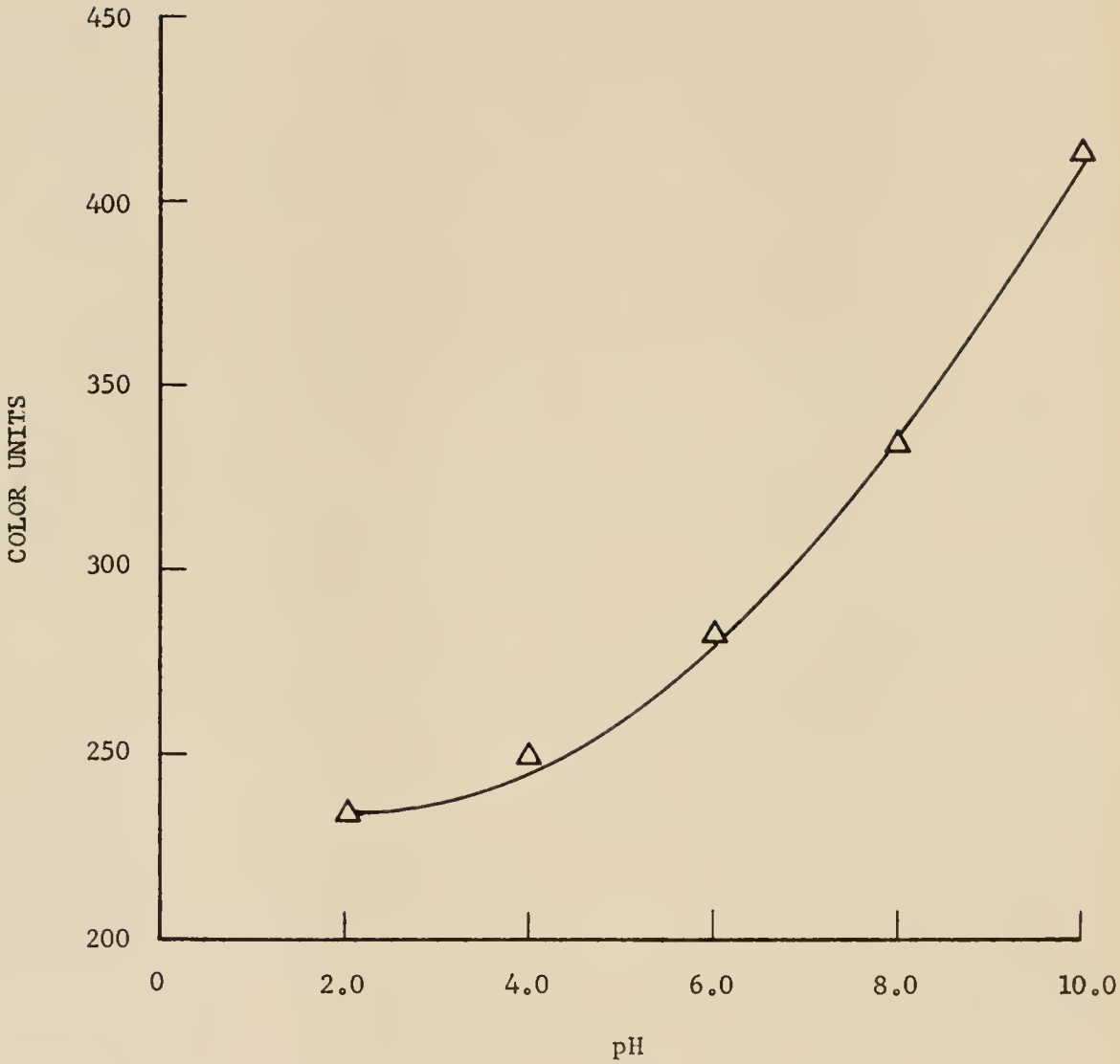


Figure 2. Effect of pH on Color of Water B

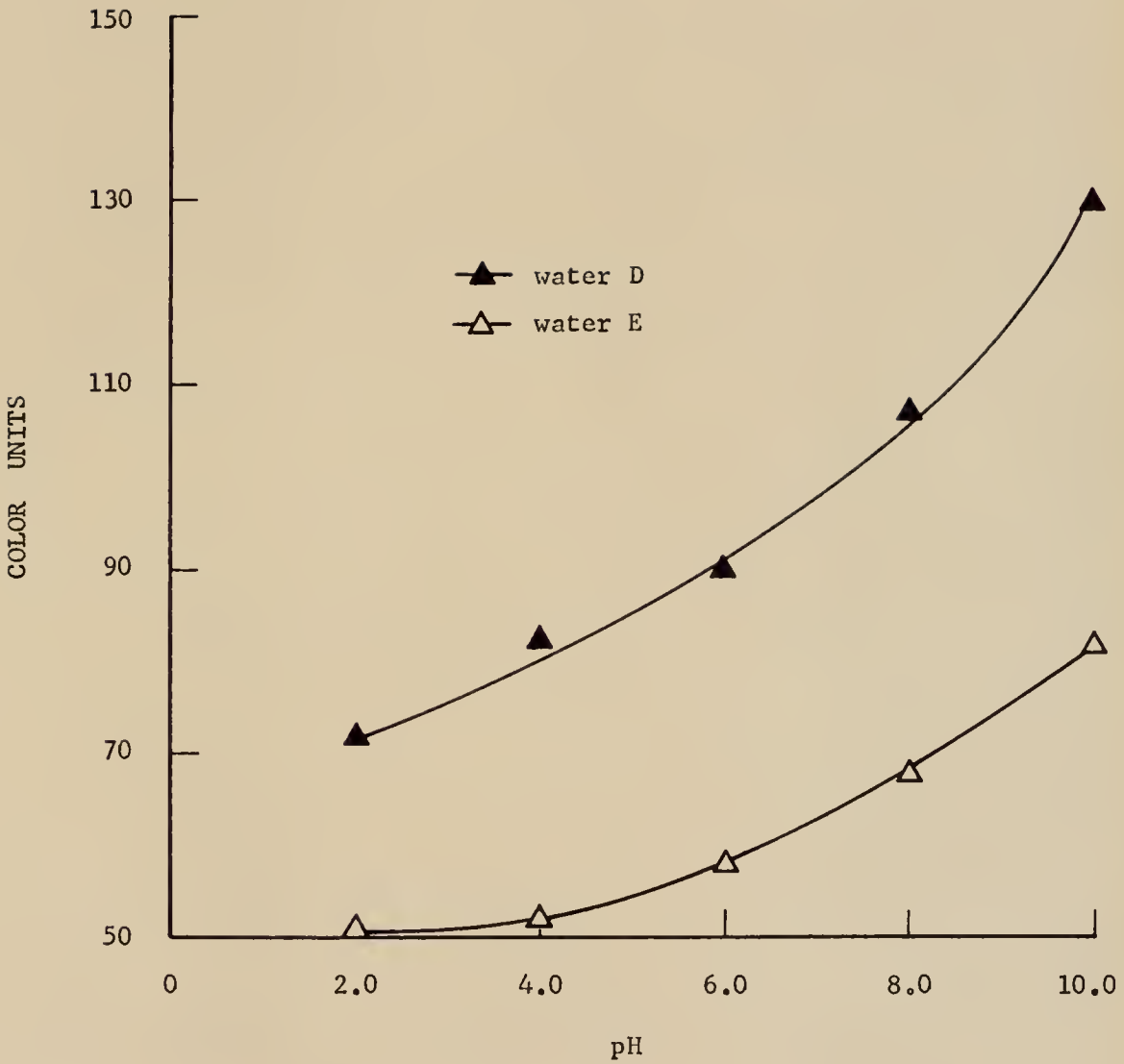


Figure 3. Effect of pH on Color of Waters D and E

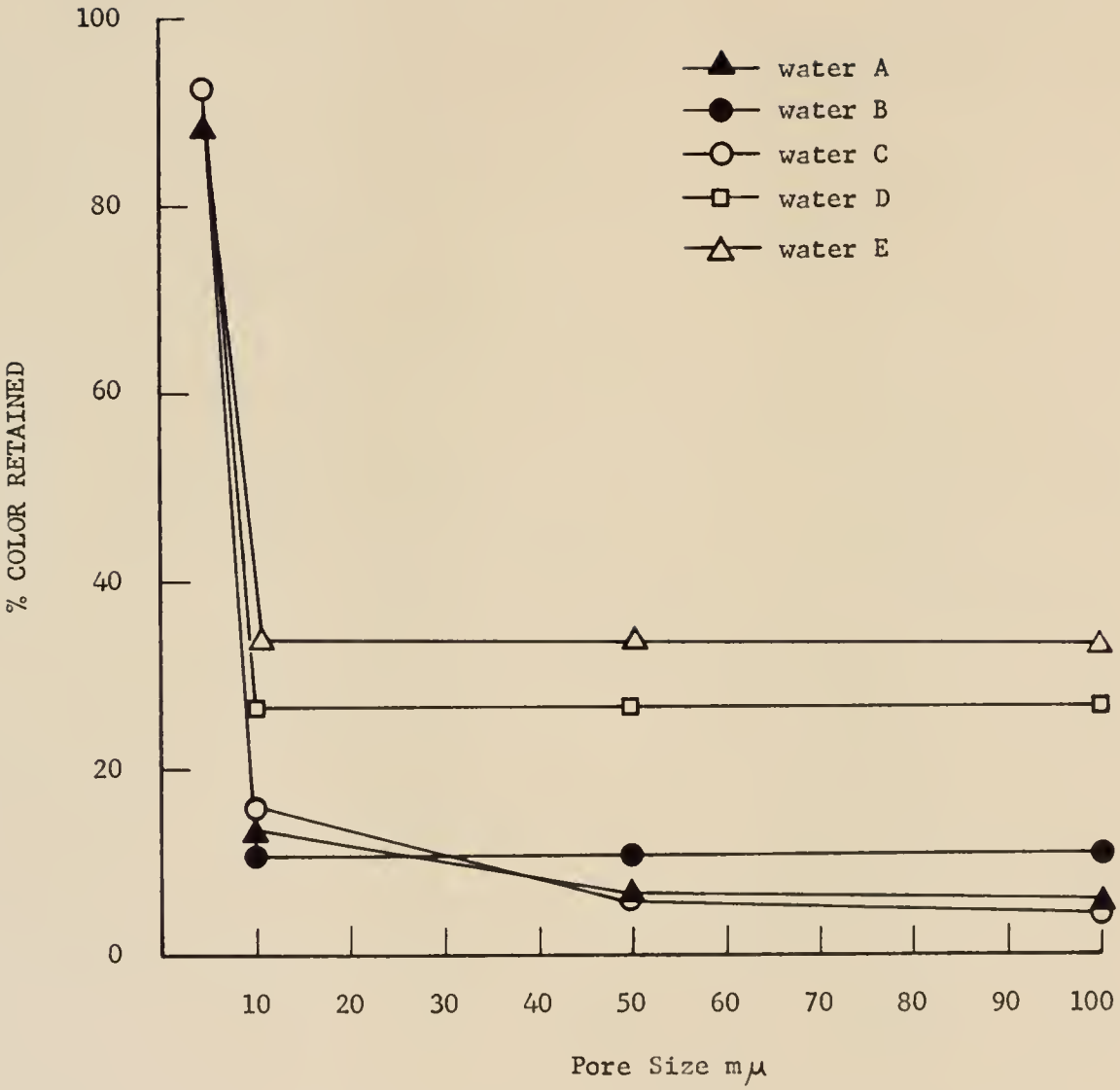


Figure 4. Dialysis and Millipore Filtration of Waters A Through E

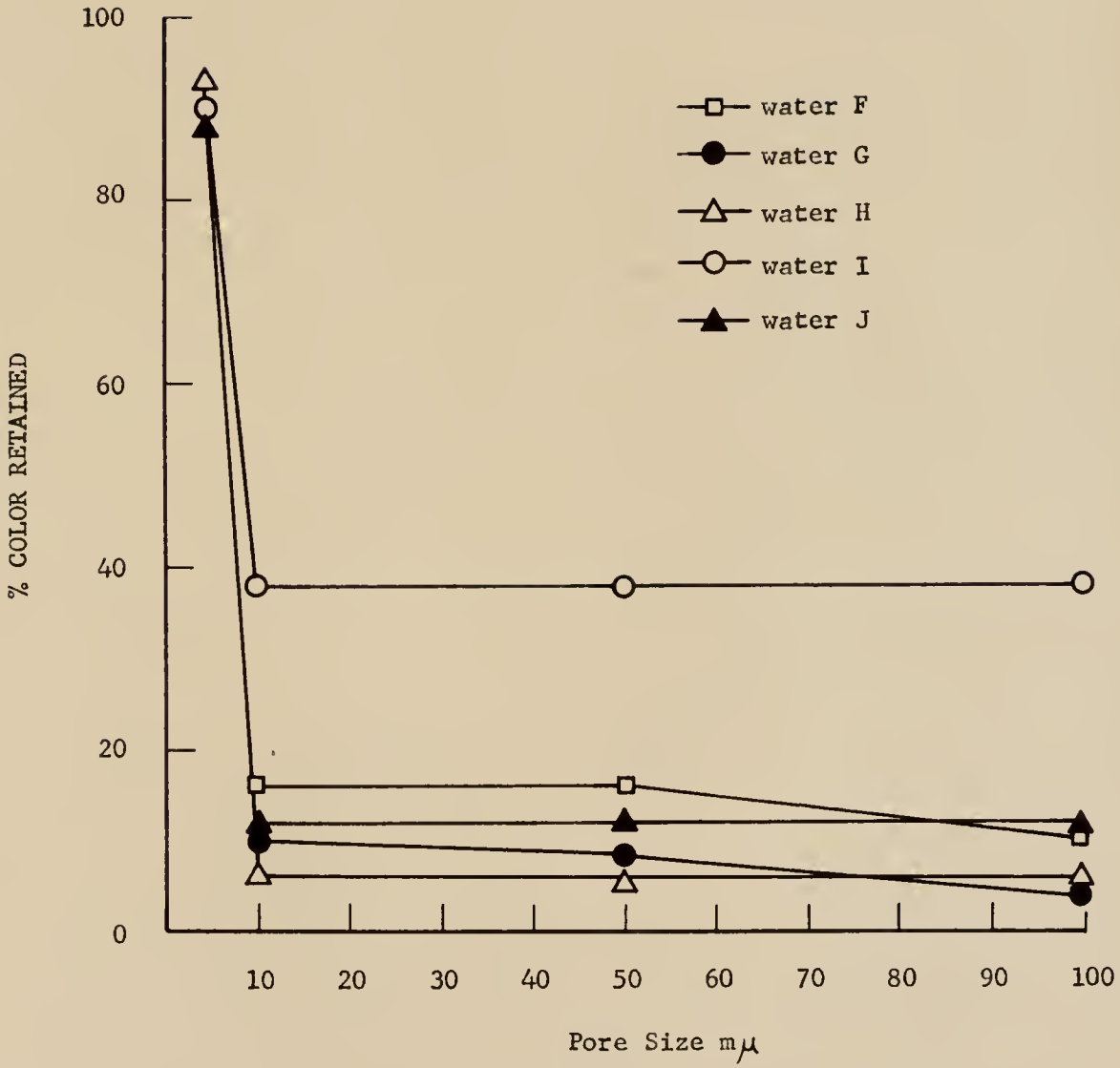


Figure 5. Dialysis and Millipore Filtration of Waters F Through J

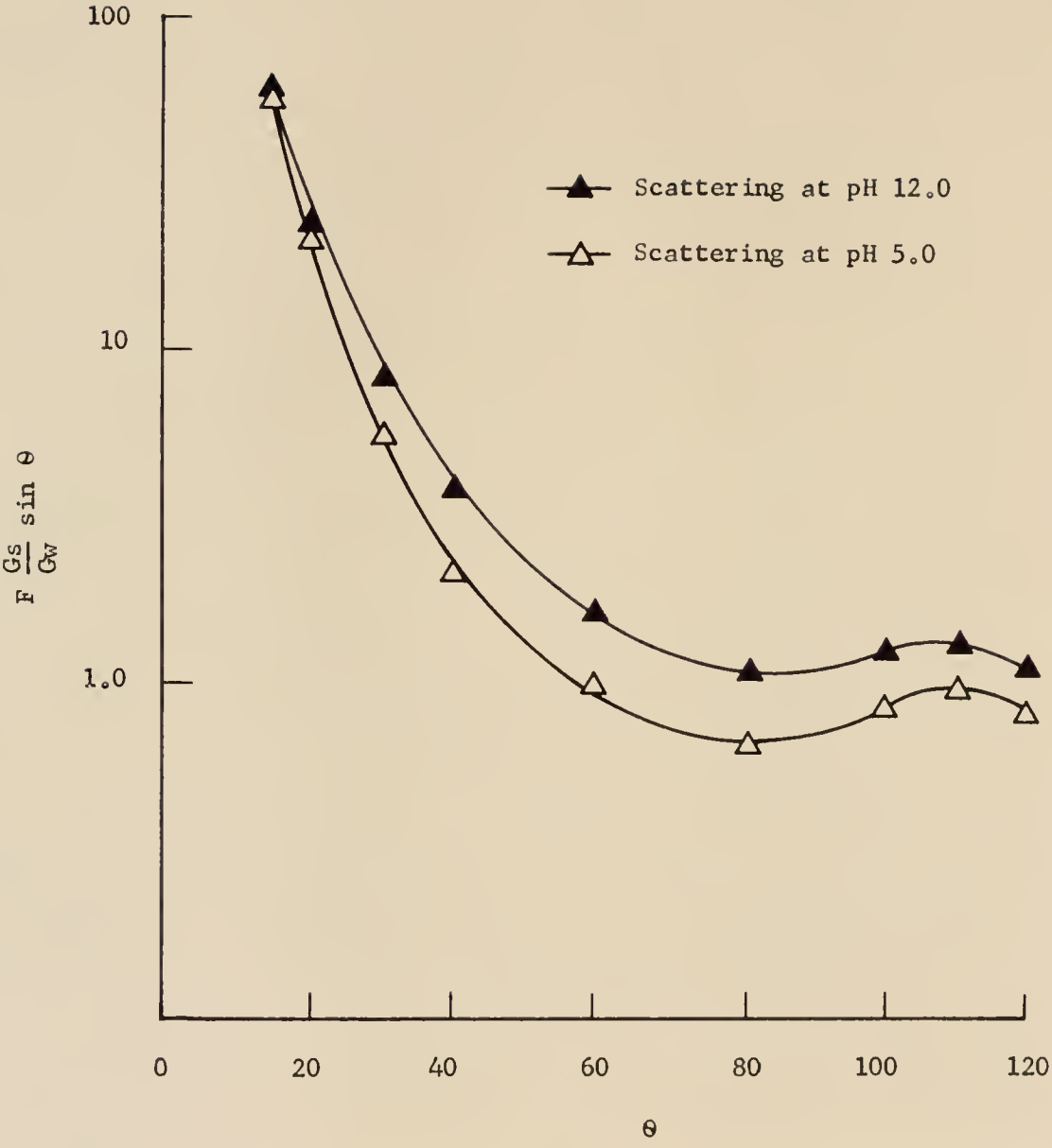


Figure 6. Light Scattering Properties of Water B at 546 mμ

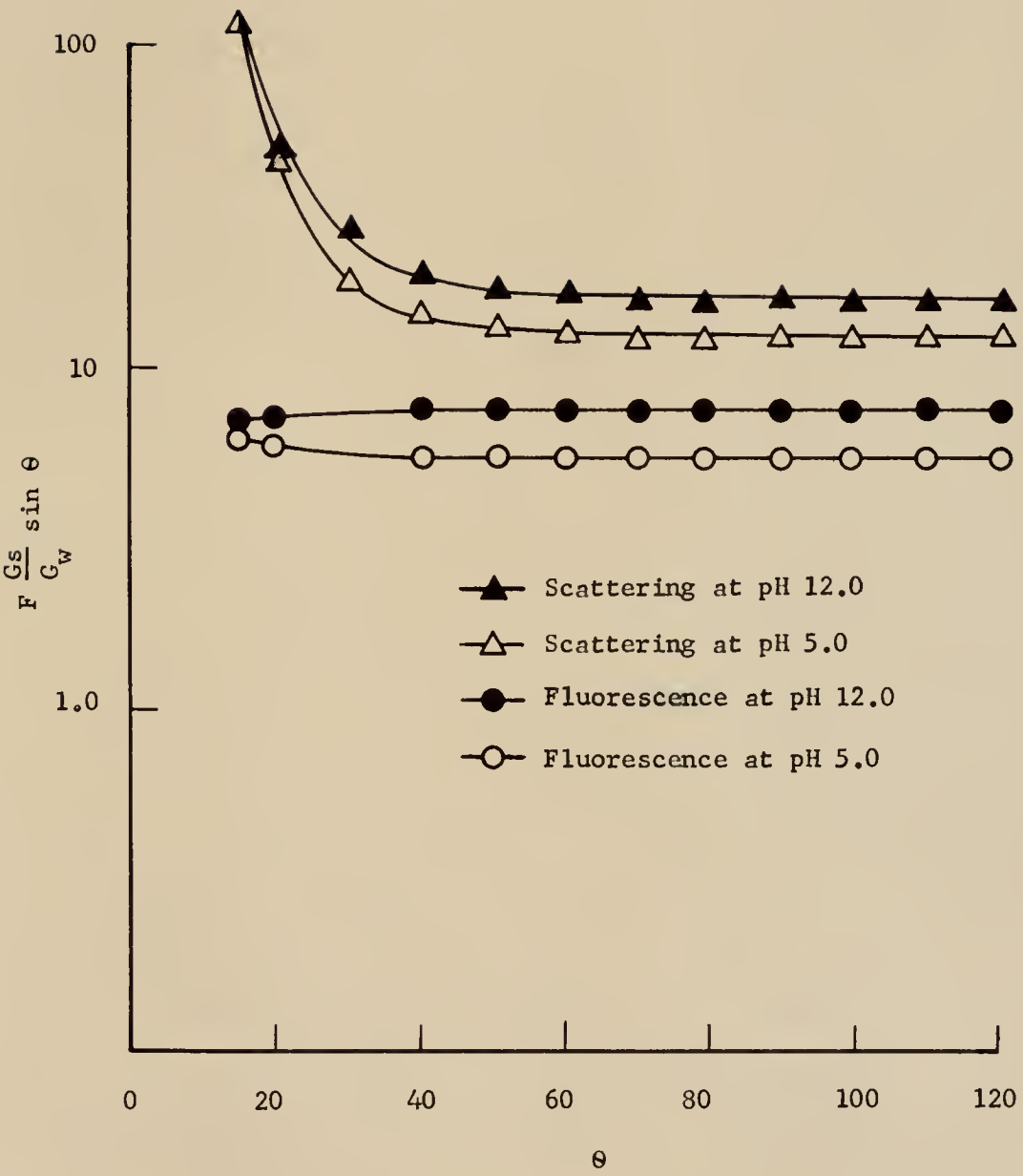


Figure 7. Light Scattering Properties of Water B at 436 mμ

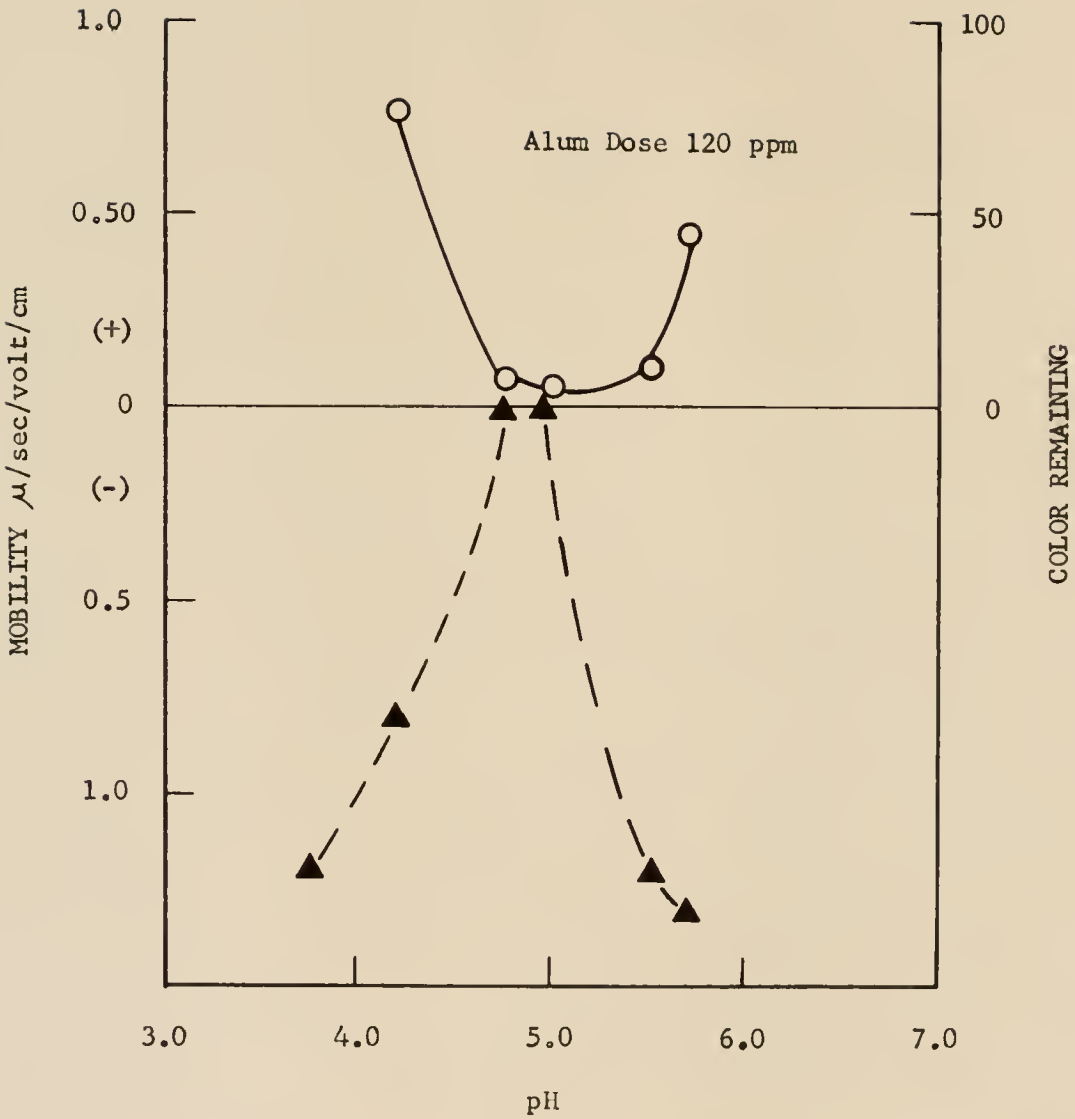


Figure 8. Effect of pH and Constant Alum Dosage on Coagulation of Water B

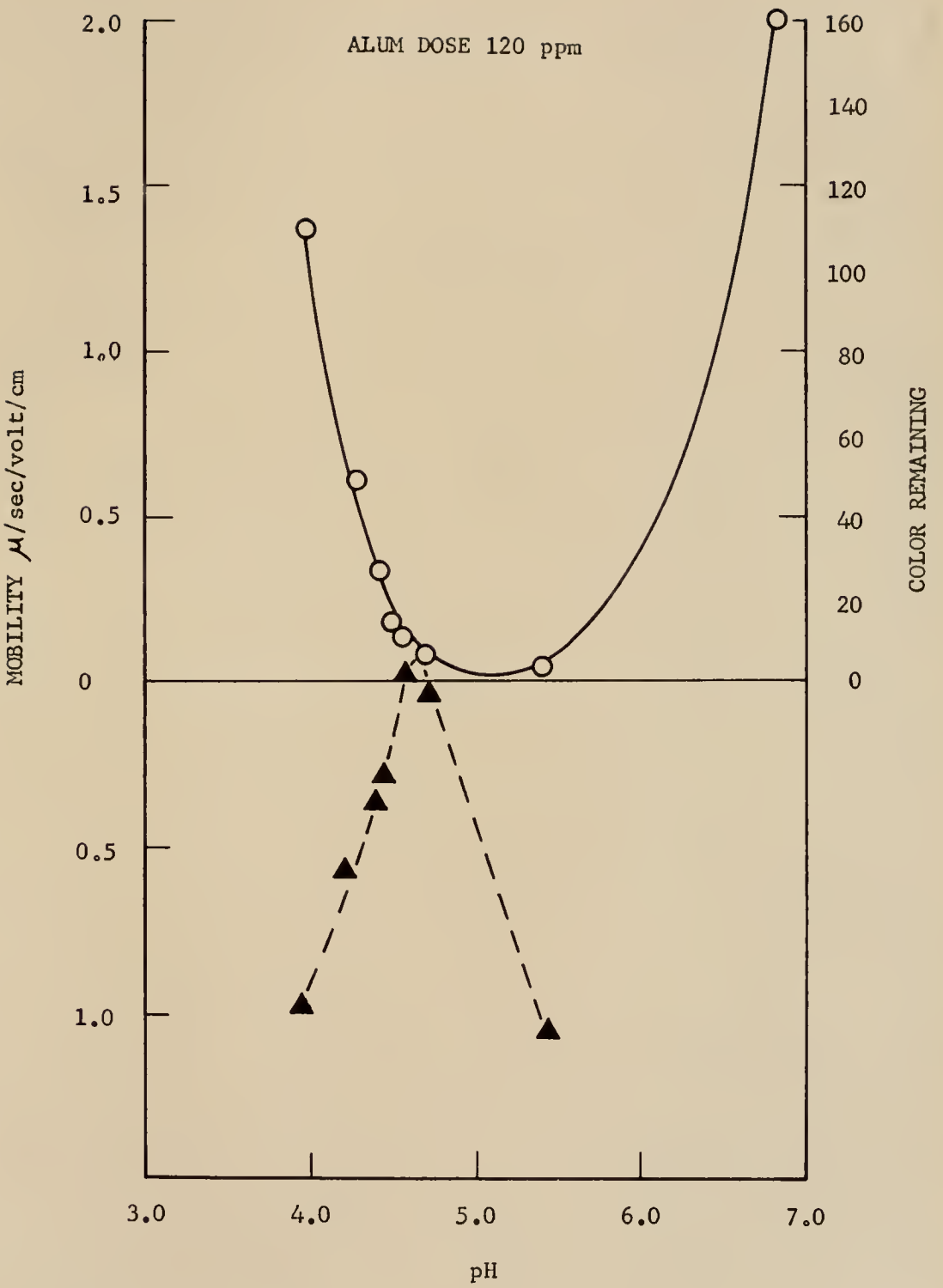


Figure 9. Effect of pH and Constant Alum Dosage on Coagulation of Water I

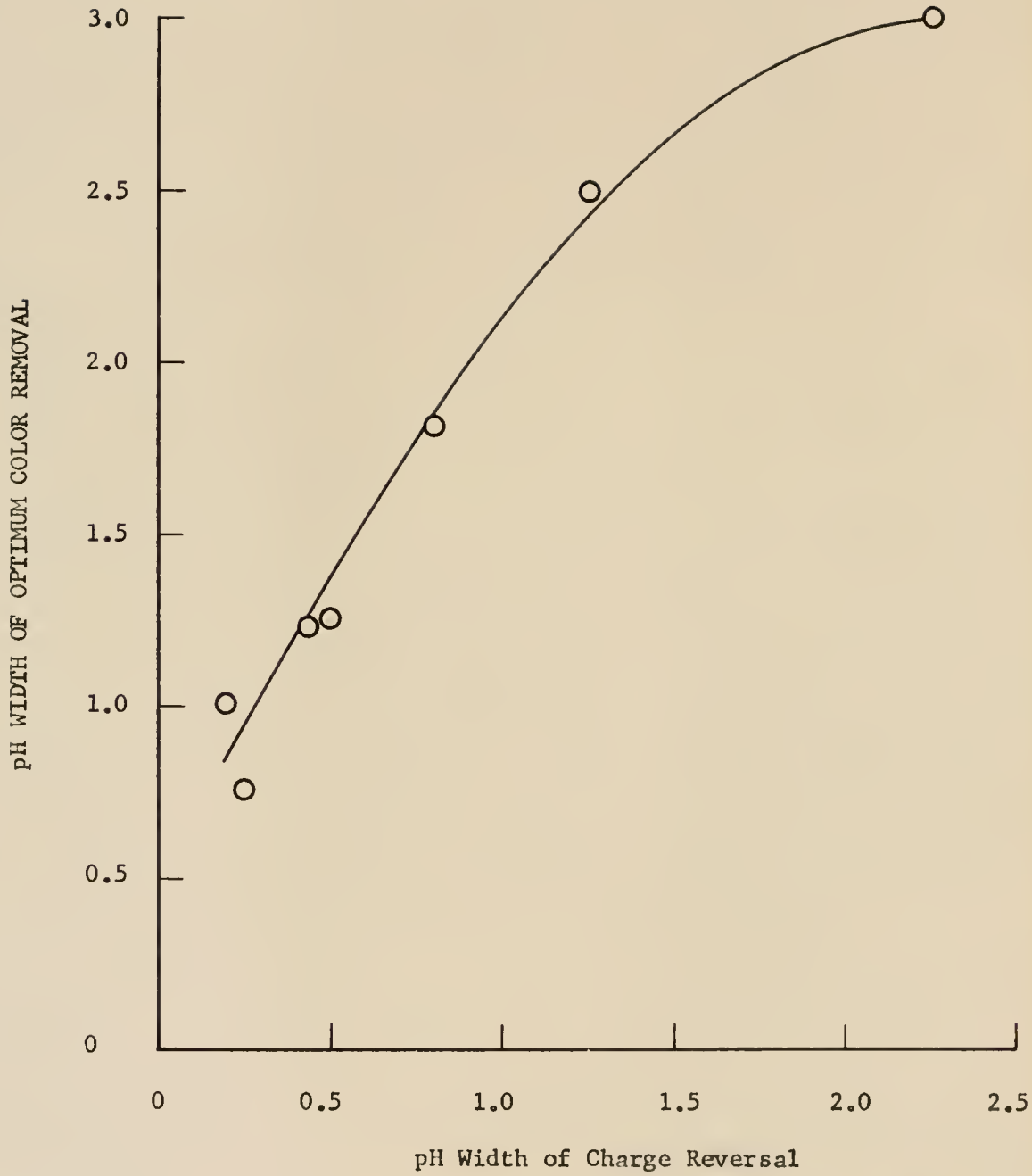


Figure 10. Variation of pH Width of Optimum Color Removal with pH Width of Charge Reversal

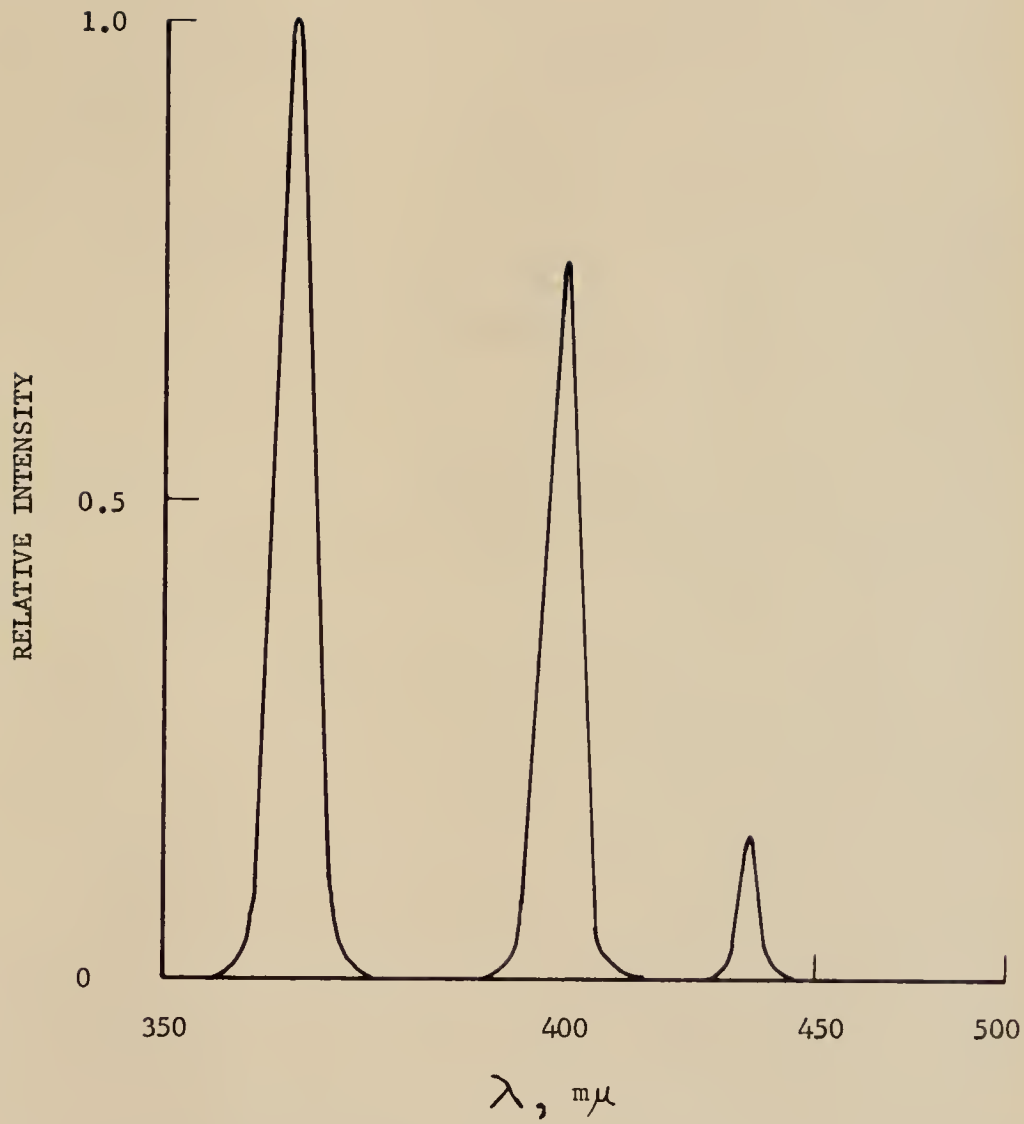


Figure 11. Spectrum of Mercury Lamp Source with Distilled Water

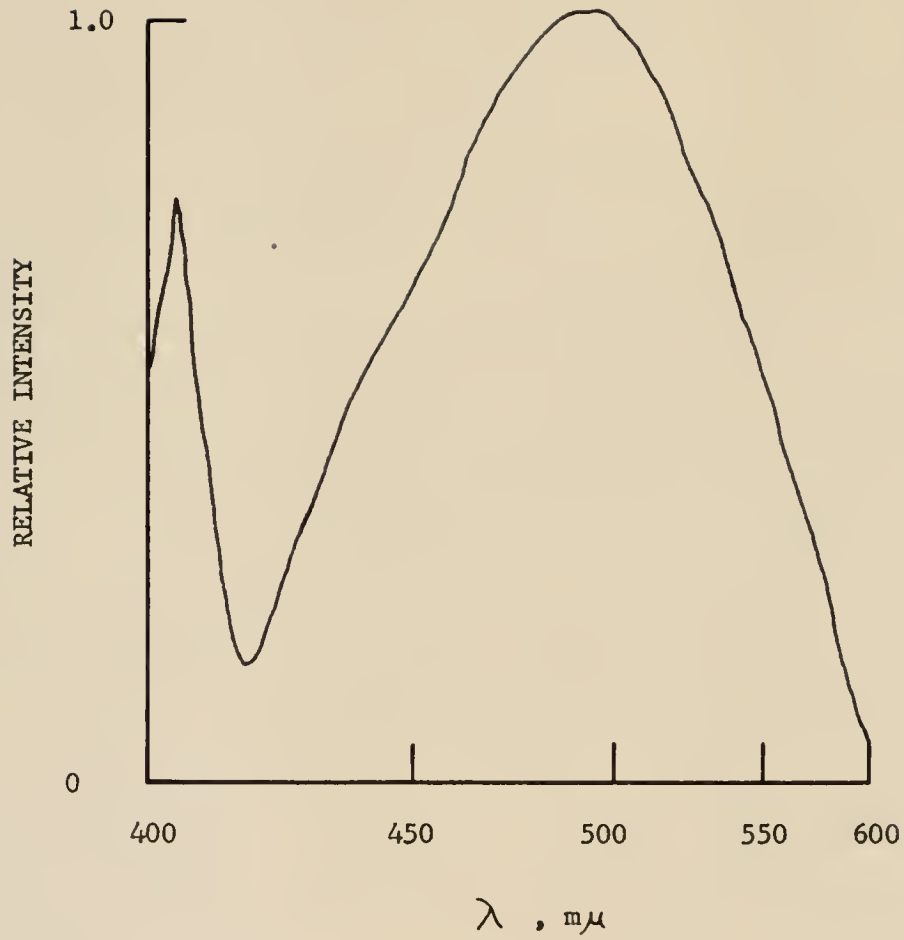


Figure 12. Fluorescence Spectrum of Water B

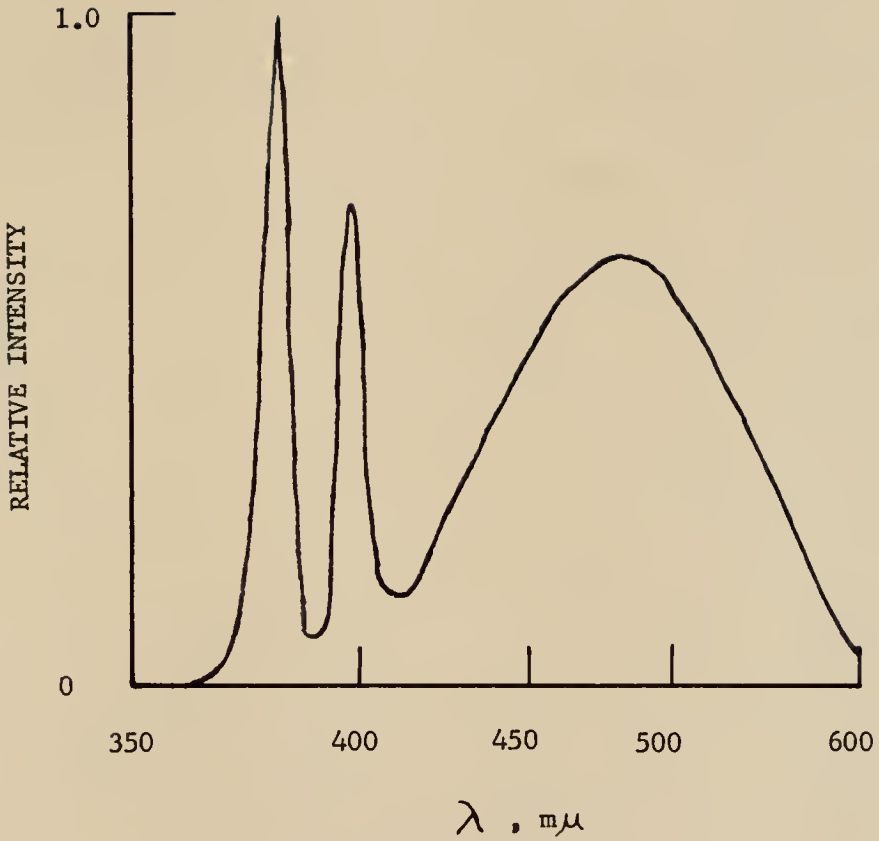


Figure 13. Fluorescence Spectrum of Water F

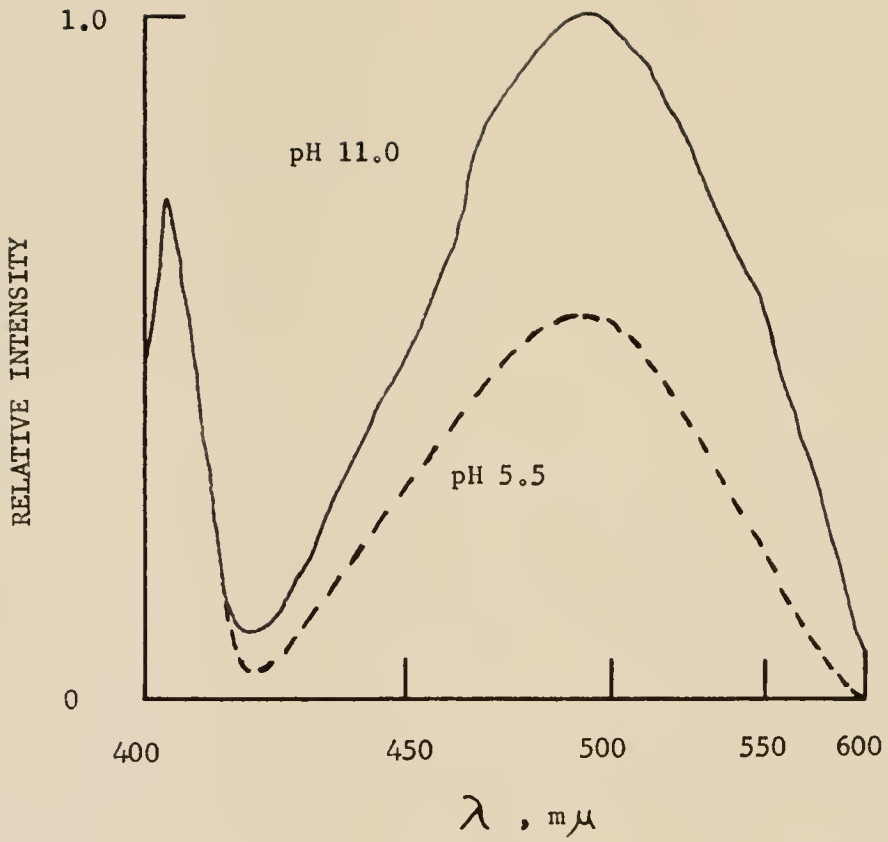


Figure 14. Effect of pH on Fluorescence Emission of Water B

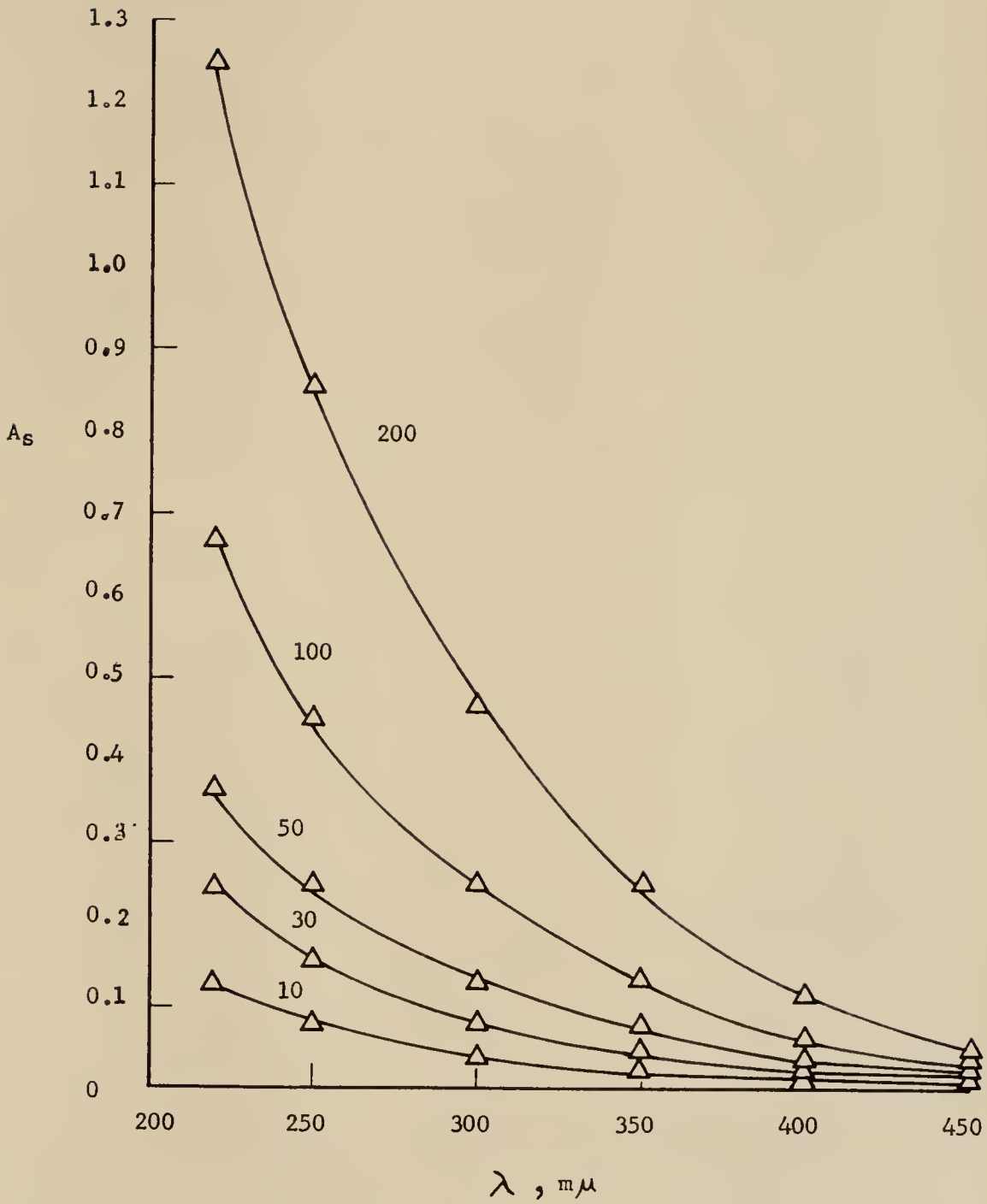


Figure 15. Ultraviolet Absorption of Water B as Function of Color Value

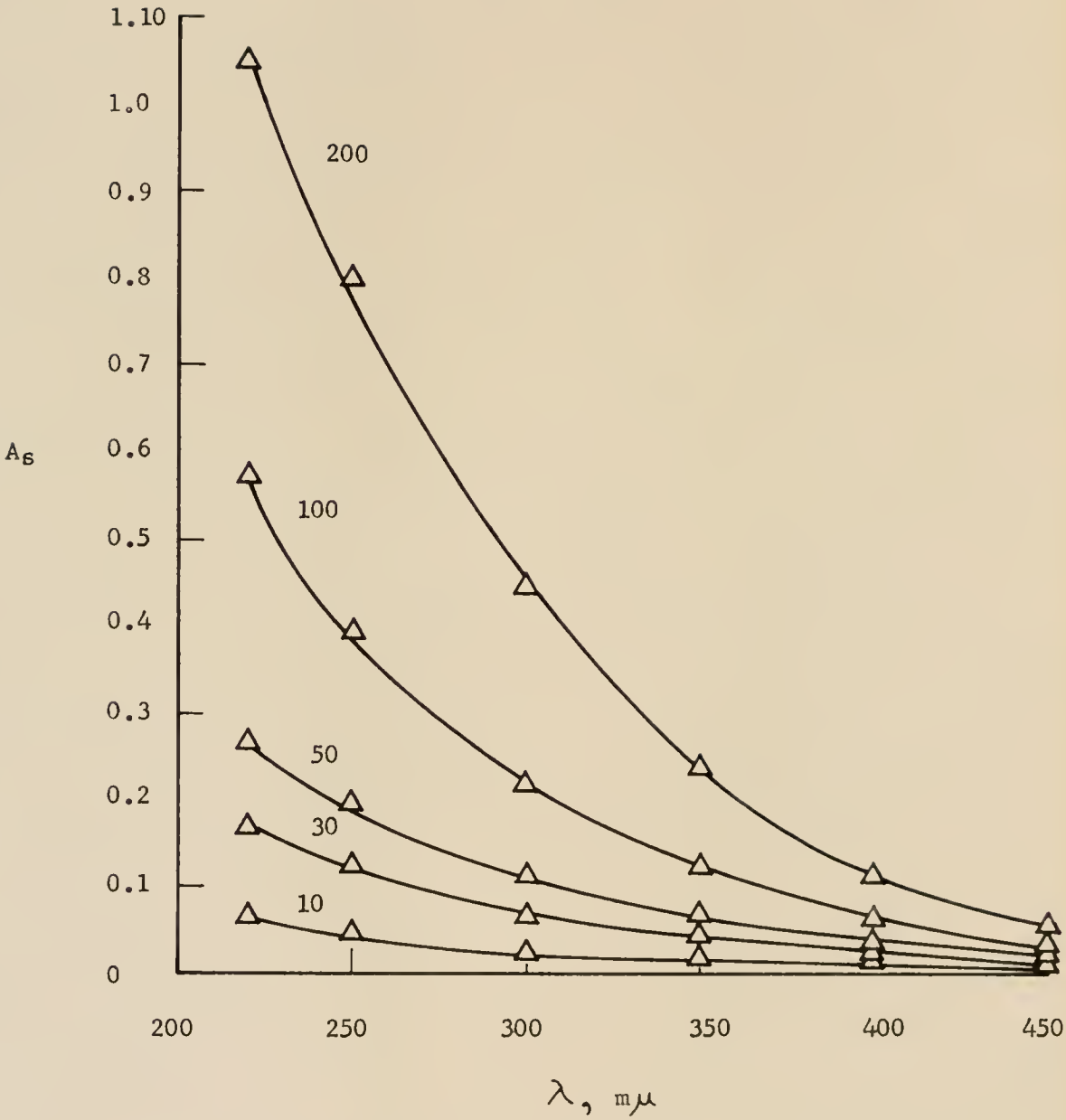


Figure 16. Ultraviolet Absorption of Water I as Function of Color Value

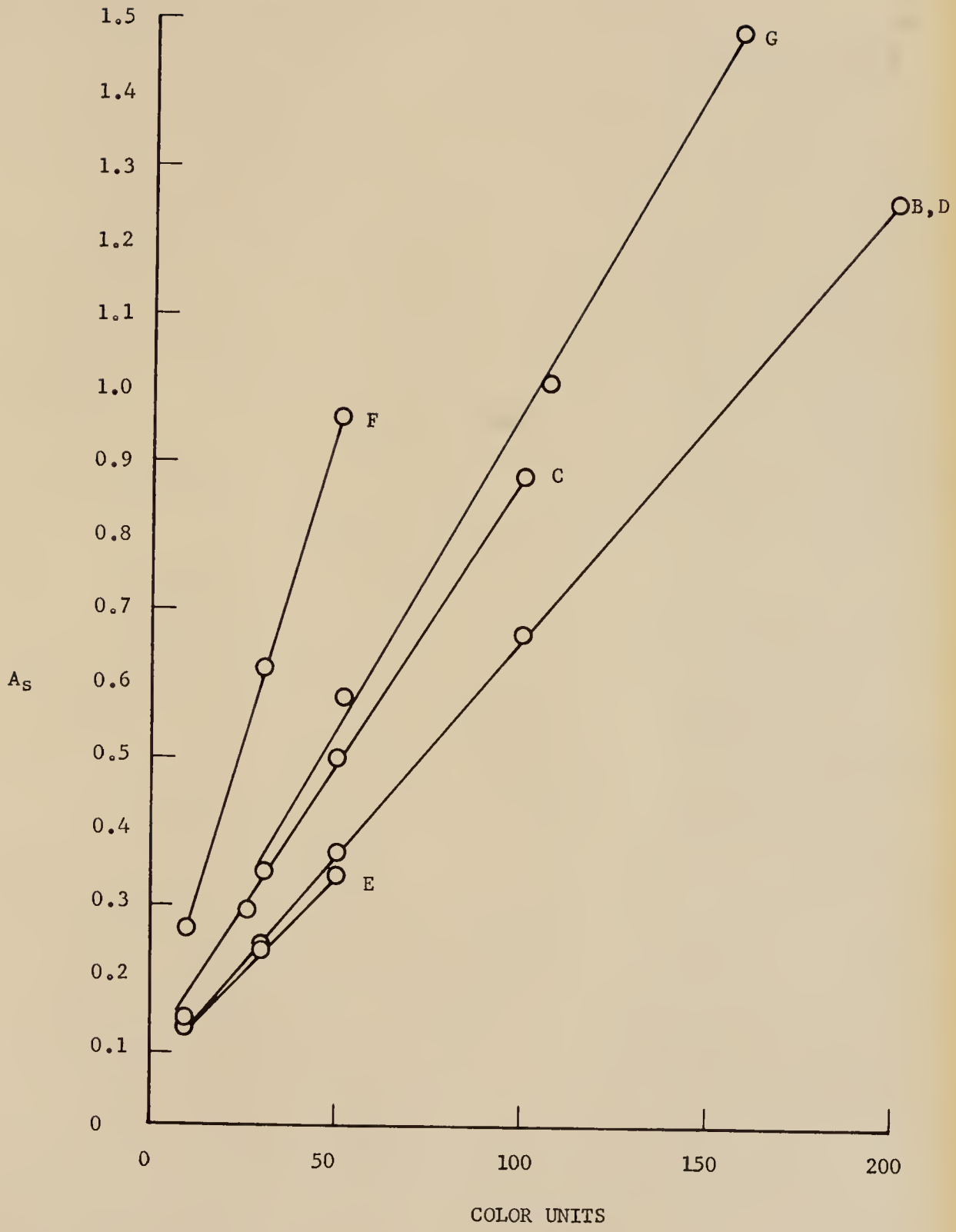


Figure 17. Absorption of Colored Waters at 220 mμ

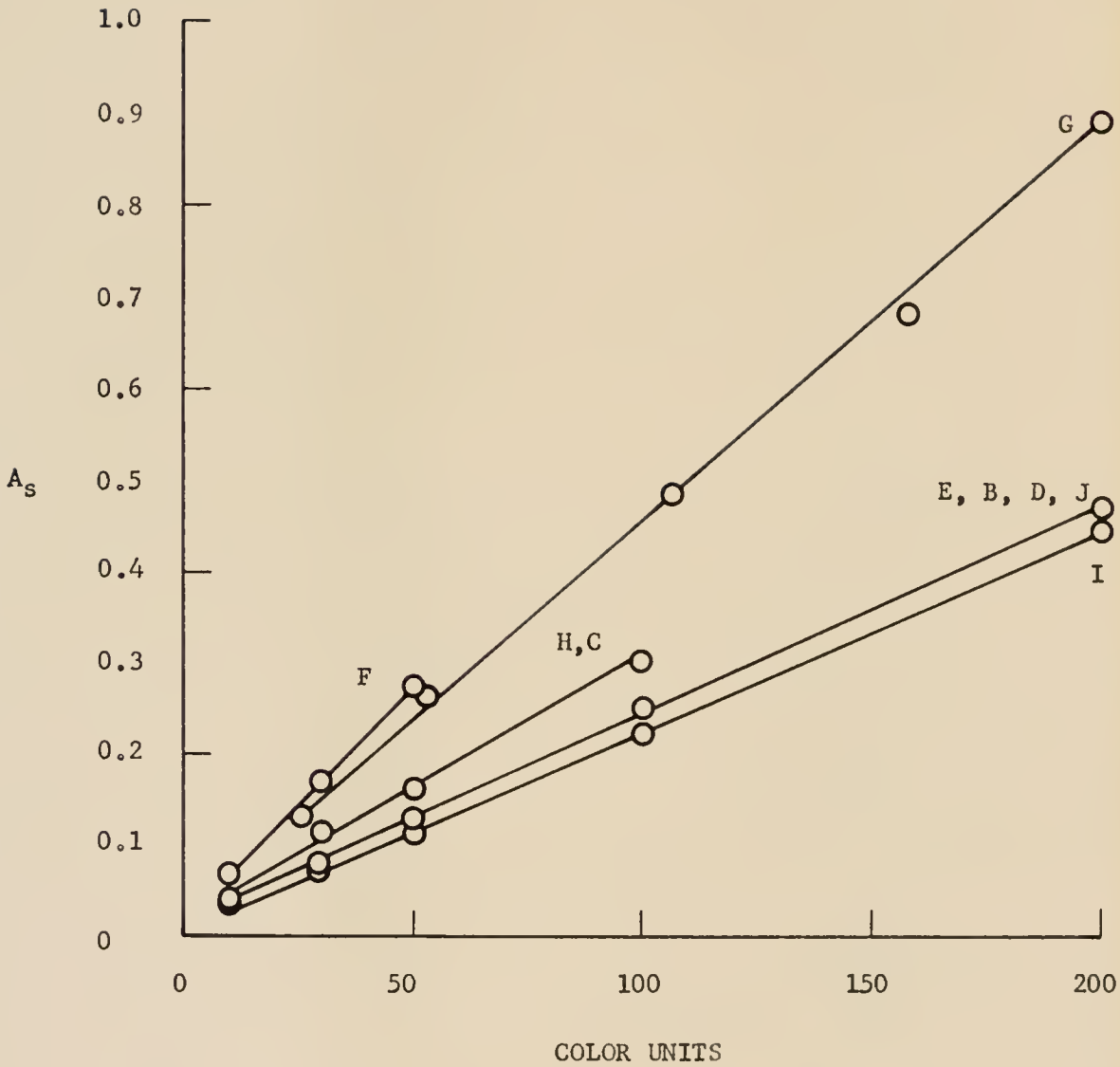


Figure 18. Absorption of Colored Waters at 300 mμ

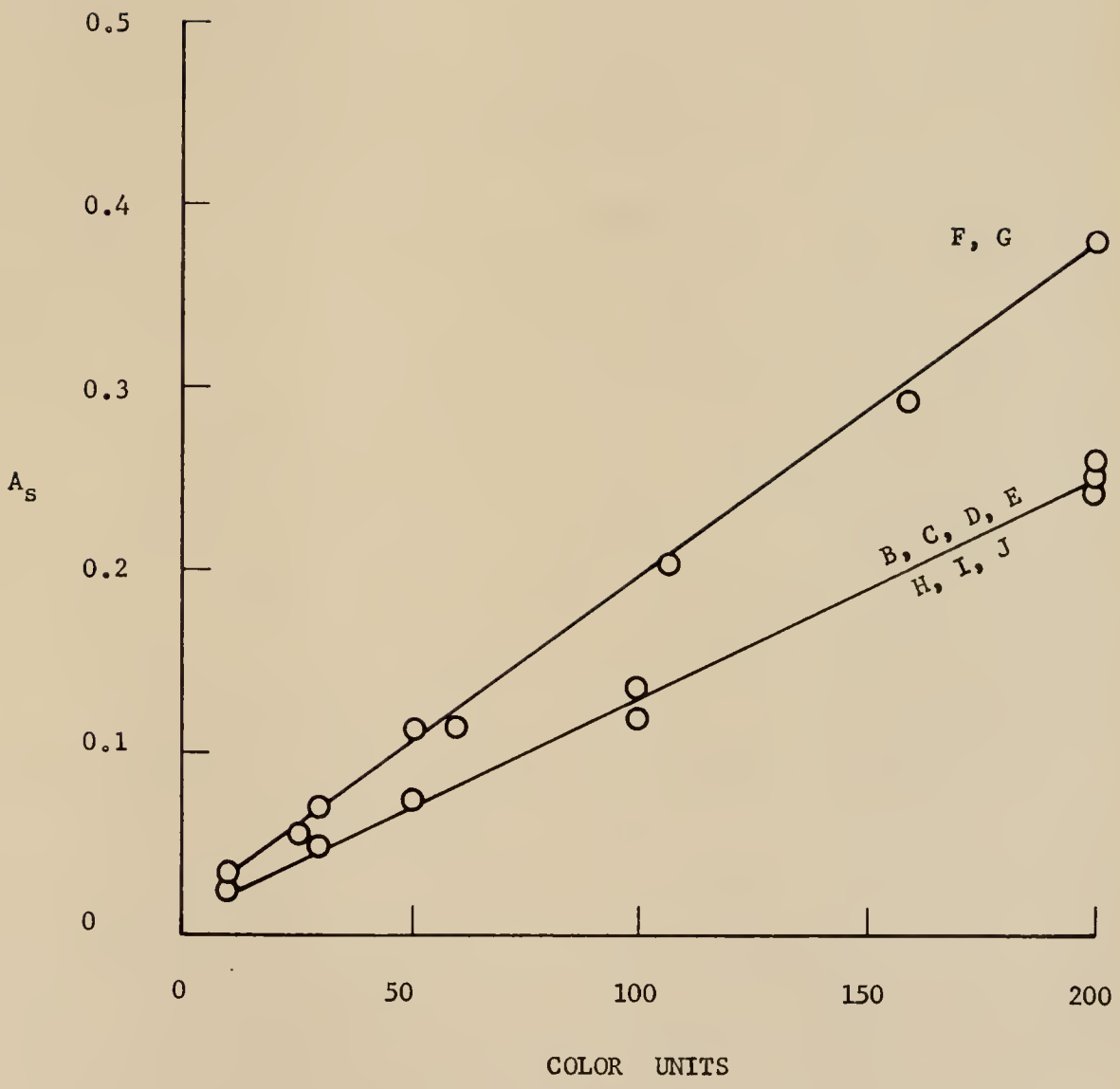


Figure 19. Absorption of Colored Waters at 350 mμ

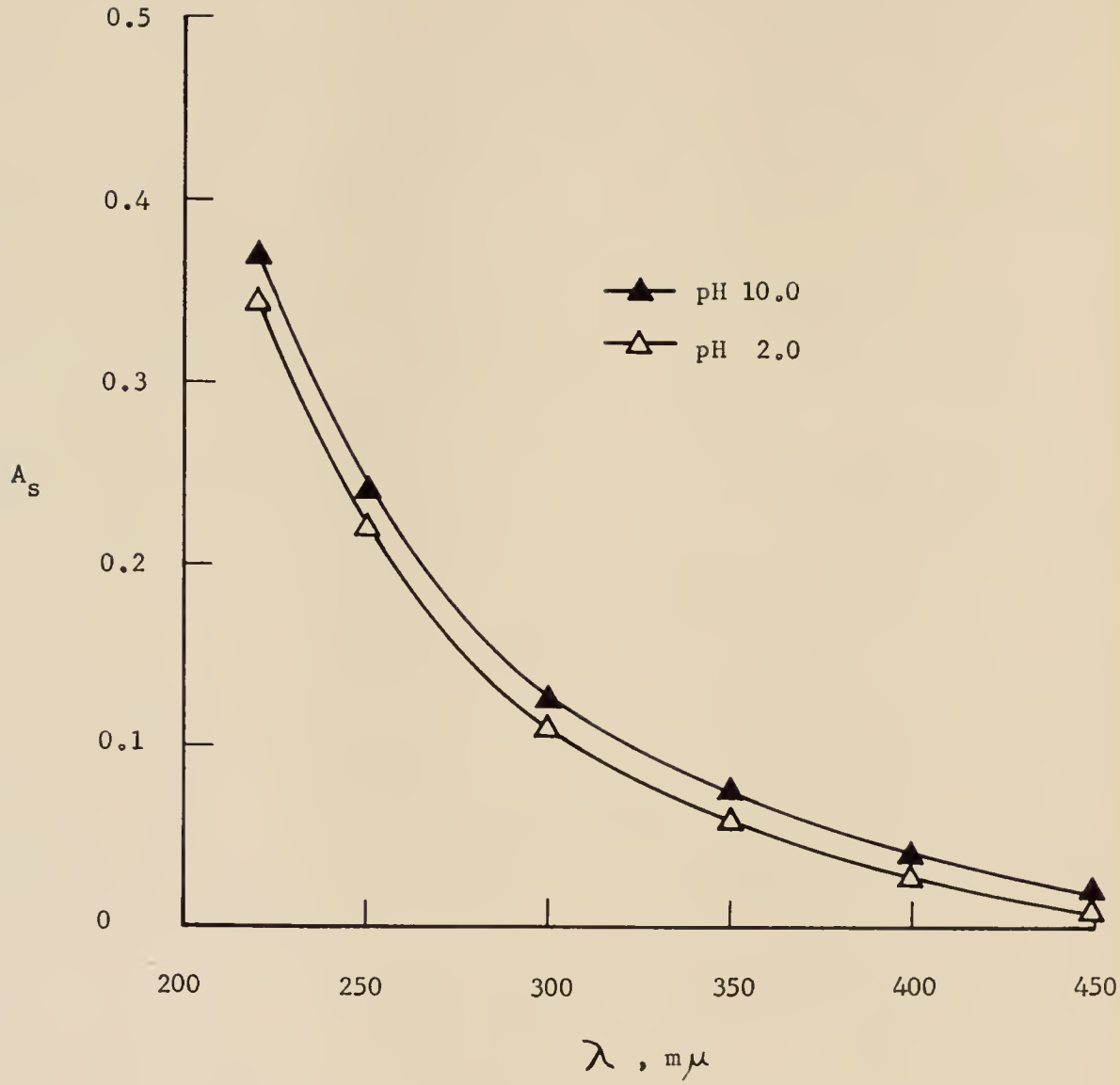


Figure 20. Effect of pH on Ultraviolet Absorption of Water B

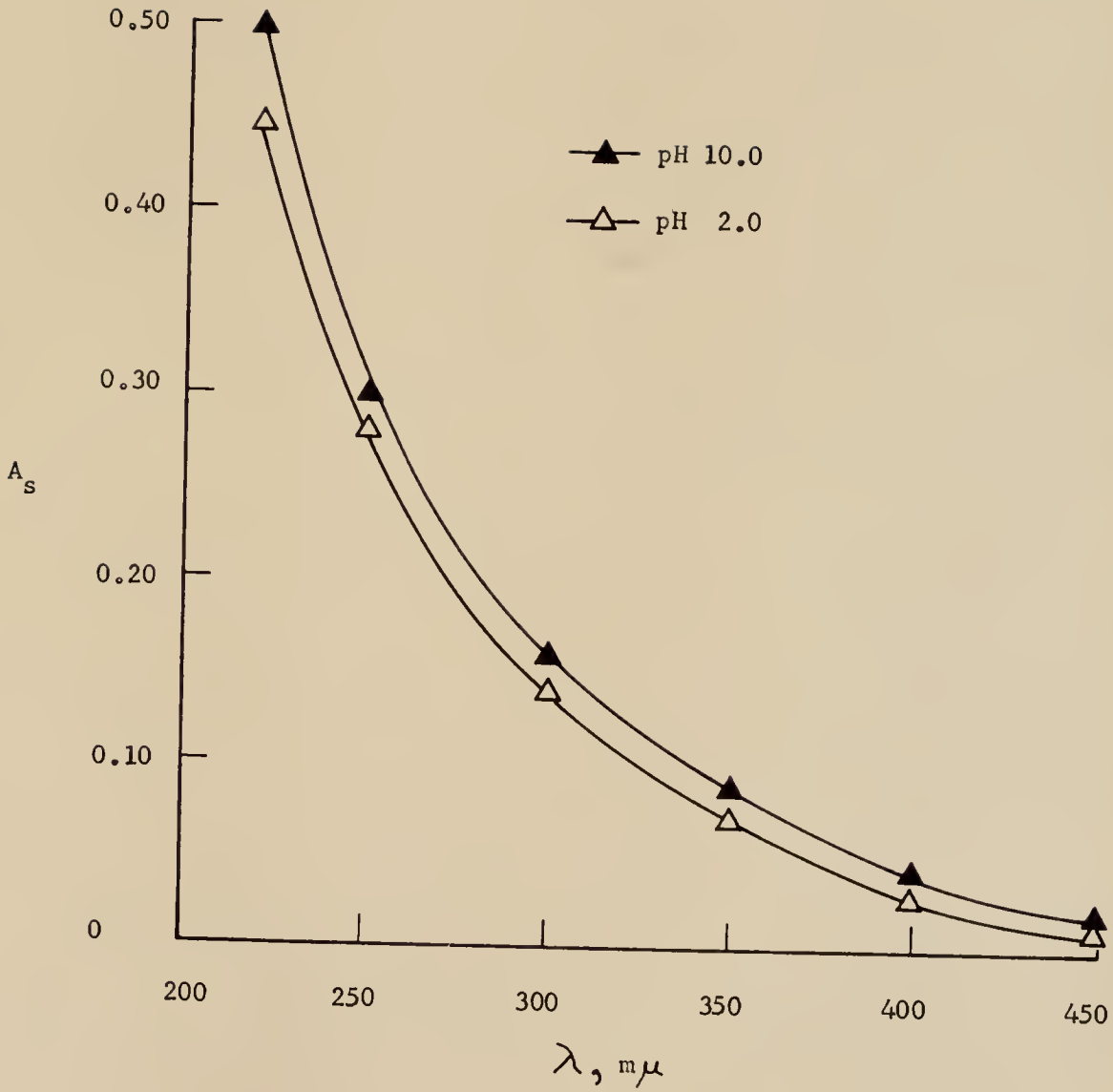


Figure 21. Effect of pH on Ultraviolet Absorption of Water C

Figure 22. Infra-Red Spectrum of "Free Acid" Fraction II

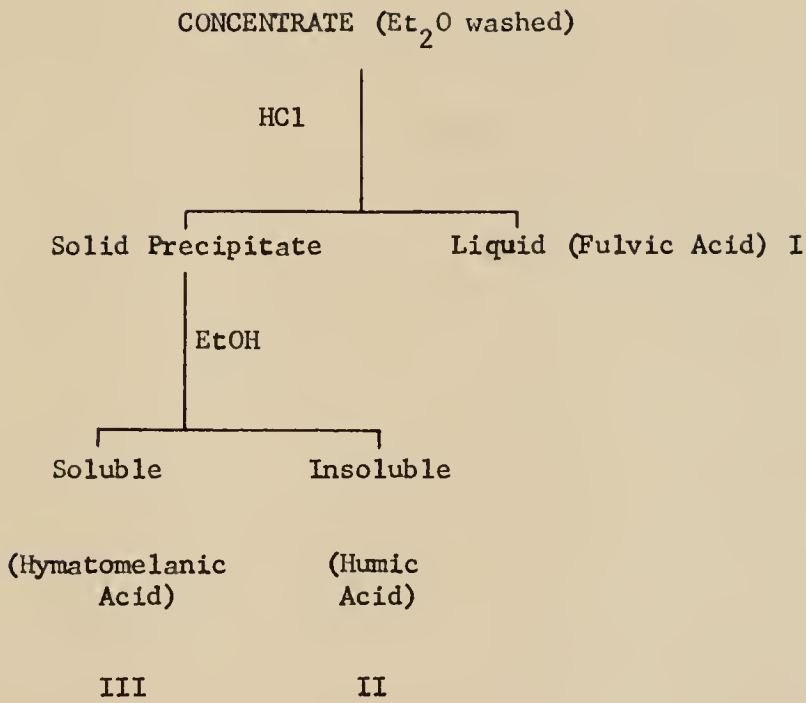


Figure 23. Fractionation Scheme

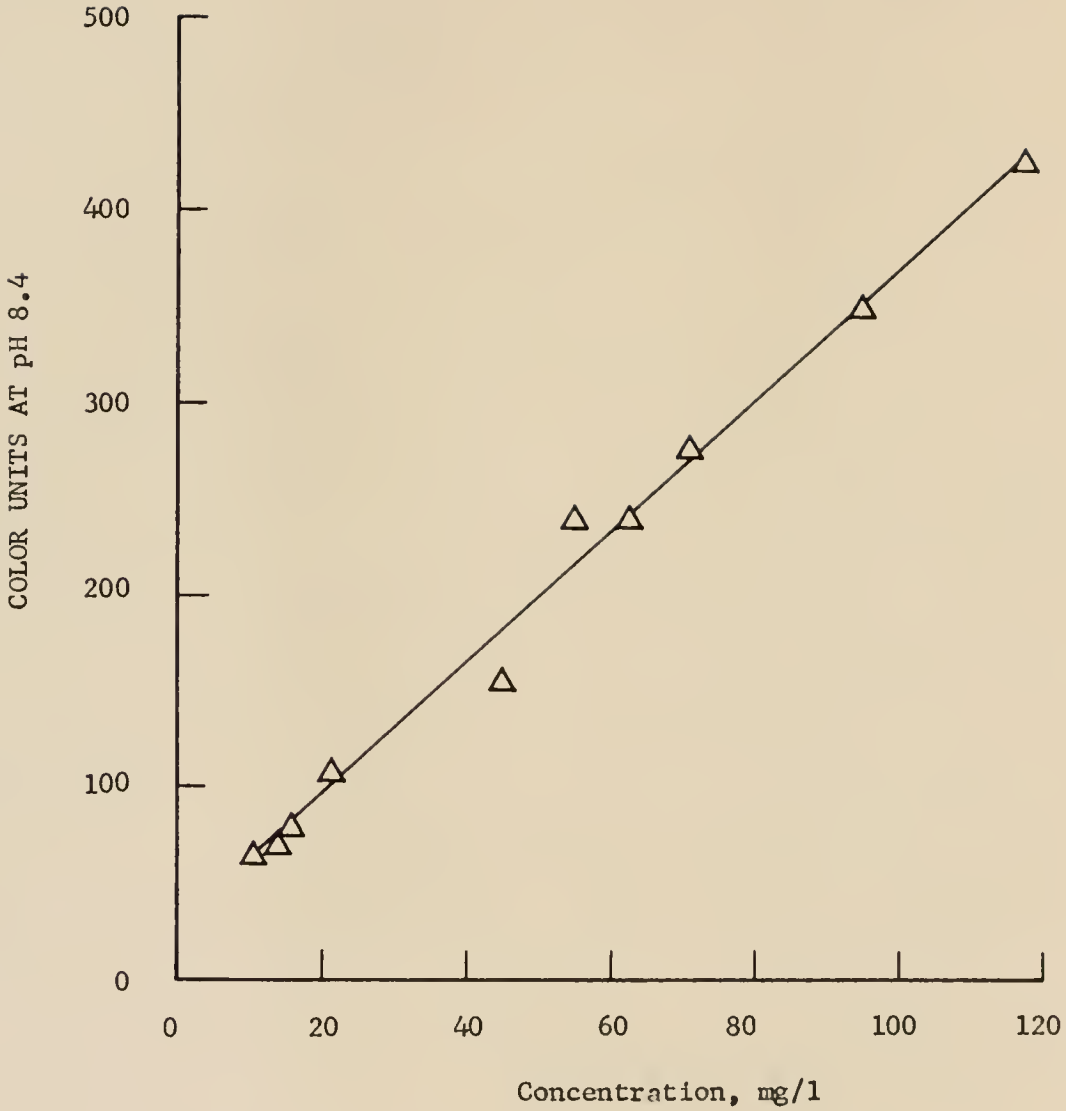


Figure 24. Relation Between Color Value and Total Organic Content of Colored Waters

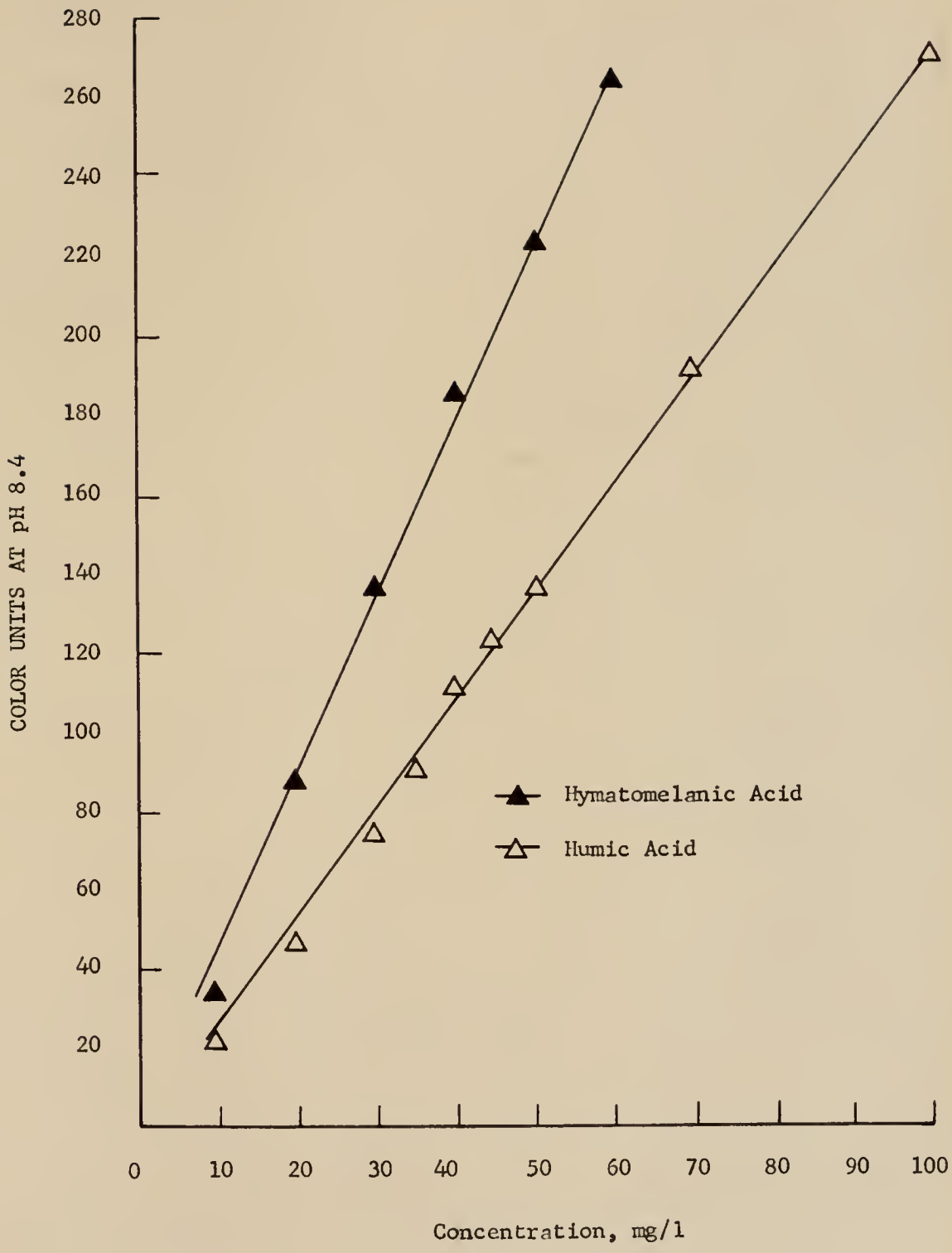


Figure 25. Relation Between Color Value and Concentration for Humic and Hymatomelanic Acid Solutions

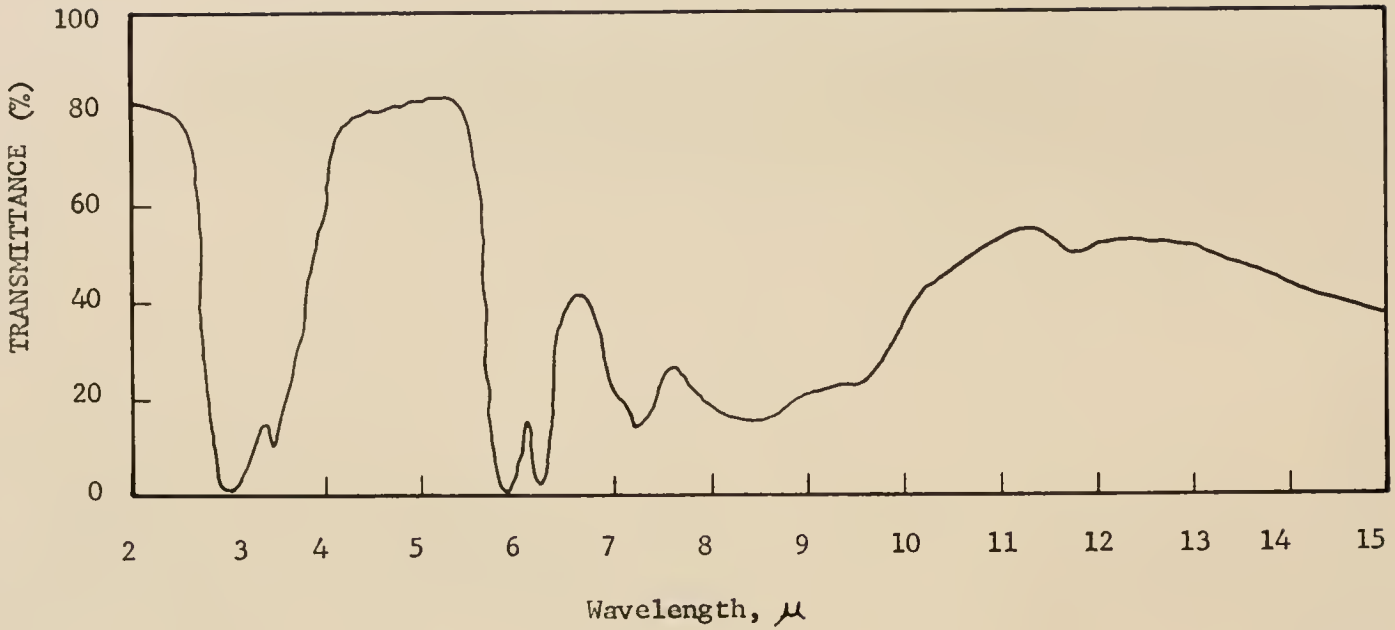


Figure 26. Infra-Red Absorption Spectrum of Fulvic Acid Fraction of Water B

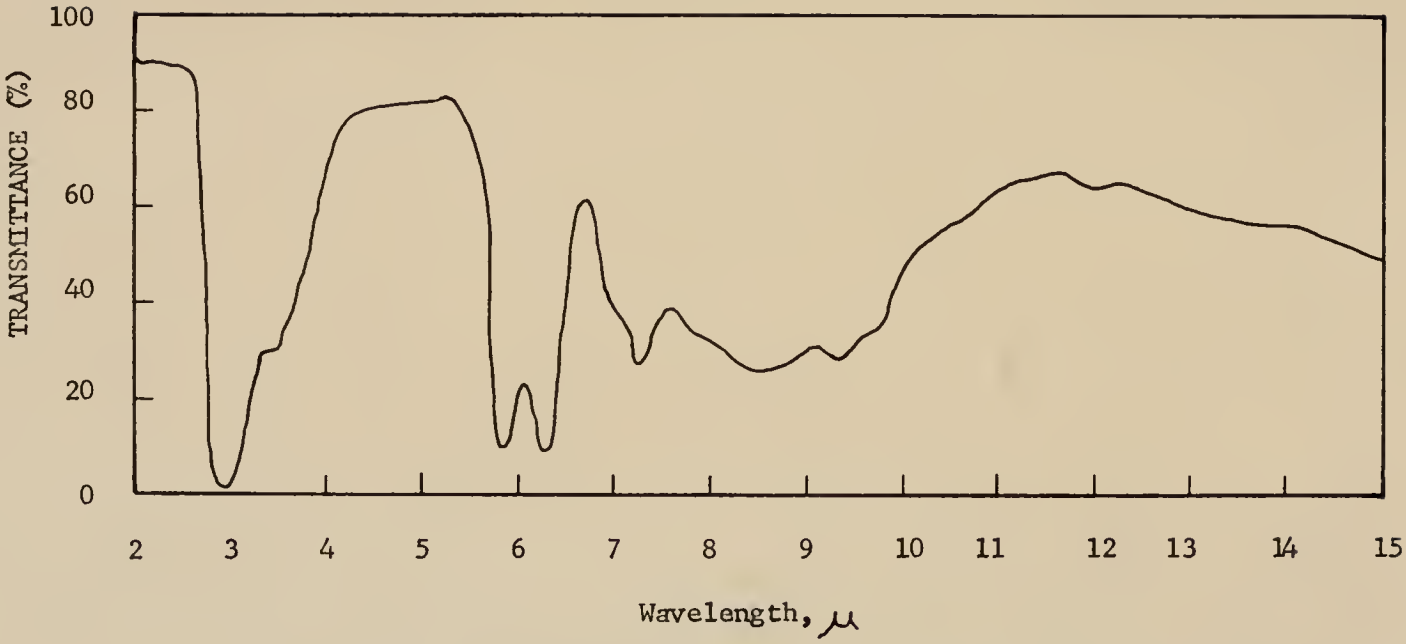


Figure 27. Infra-Red Absorption Spectrum of Fulvic Acid Fraction of Water C

Figure 28. Infra-Red Absorption Spectrum for Methyl Ester of Fulvic Acid from Water A

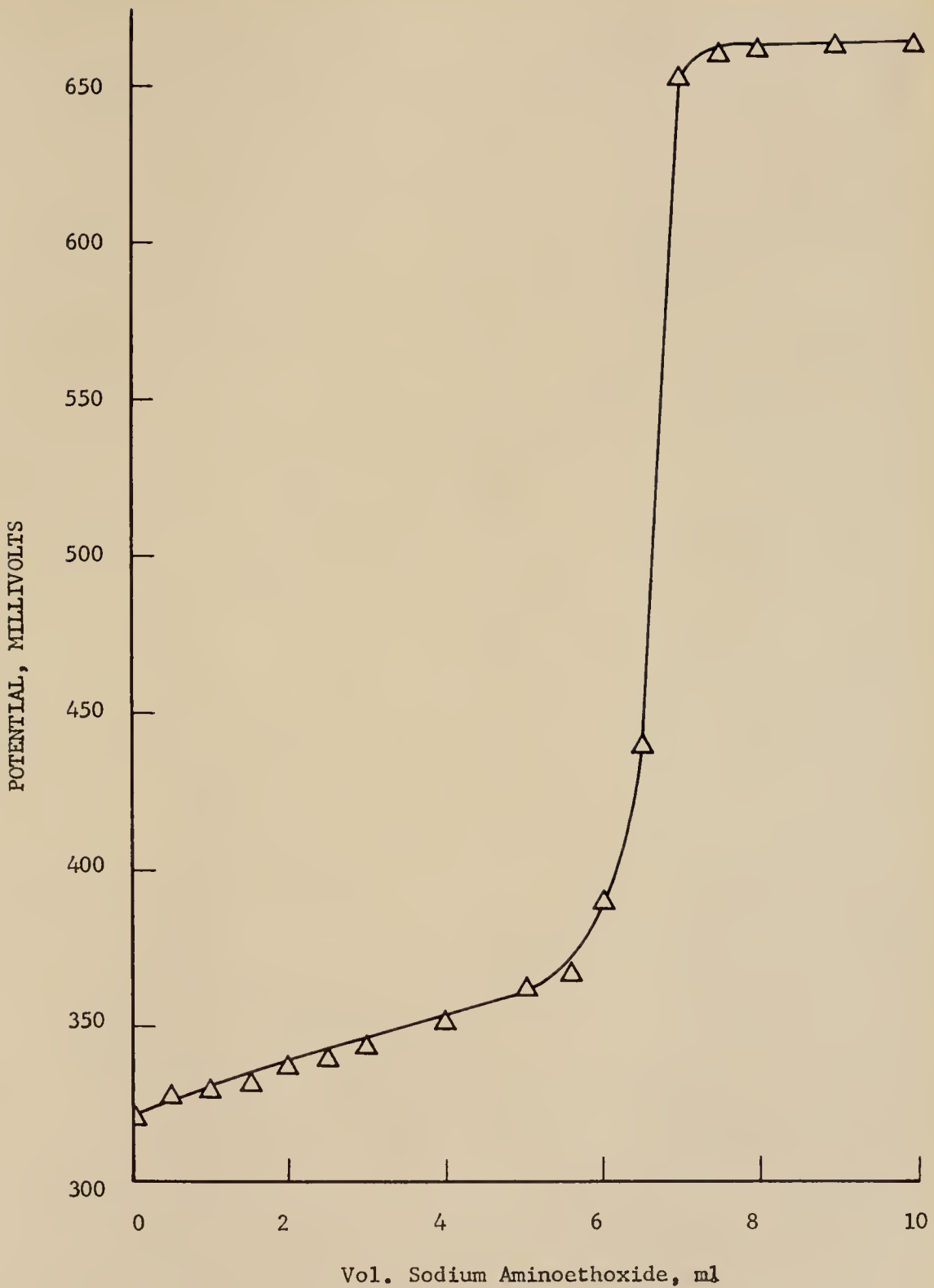


Figure 29. Potentiometric Titration of Benzoic Acid in Ethylenediamine

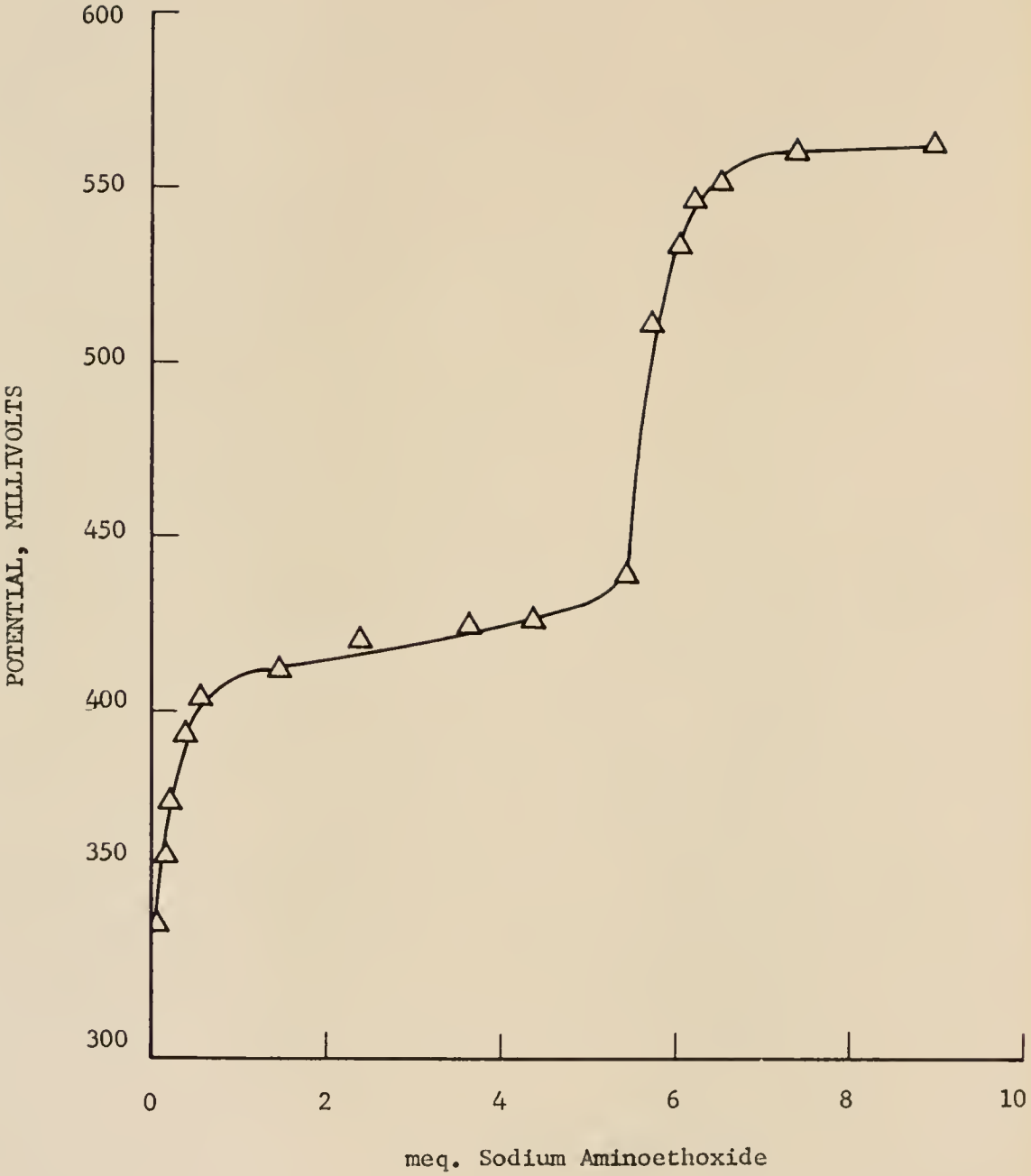


Figure 30. Potentiometric Titration of Phenol in Ethylene-diamine

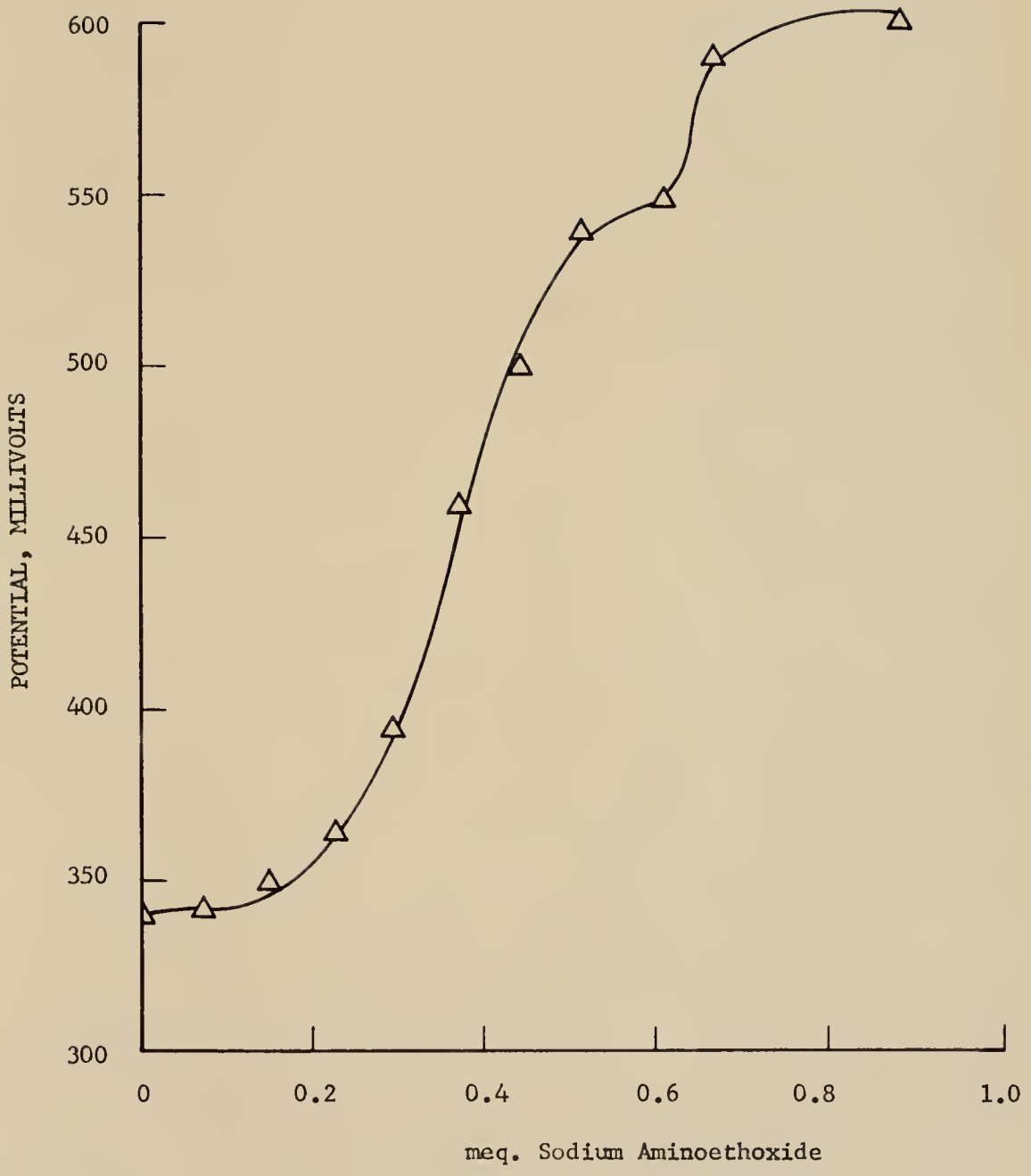


Figure 31. Potentiometric Titration of Resorcinol in Ethylenediamine

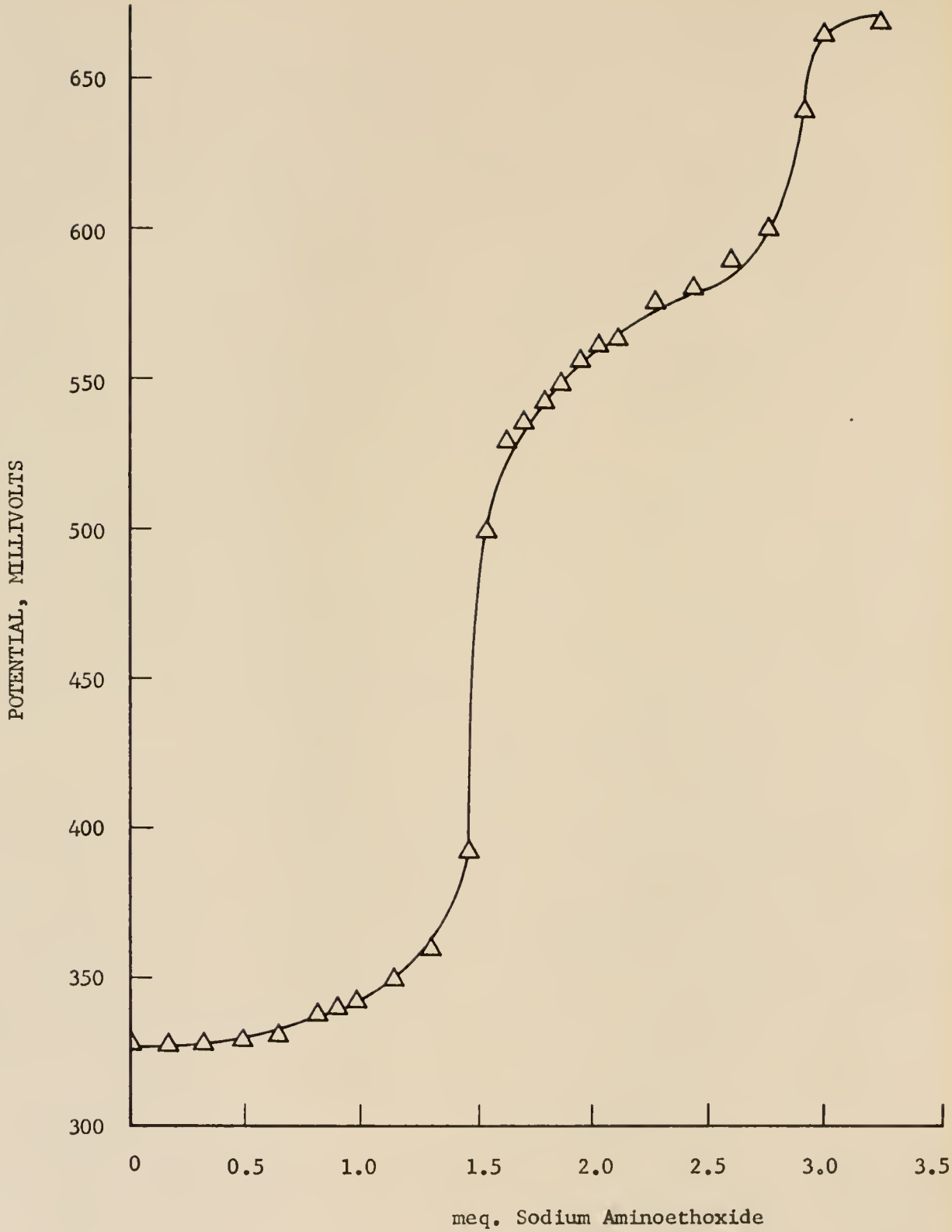


Figure 32. Potentiometric Titration of o-Hydroxybenzoic Acid in Ethylenediamine

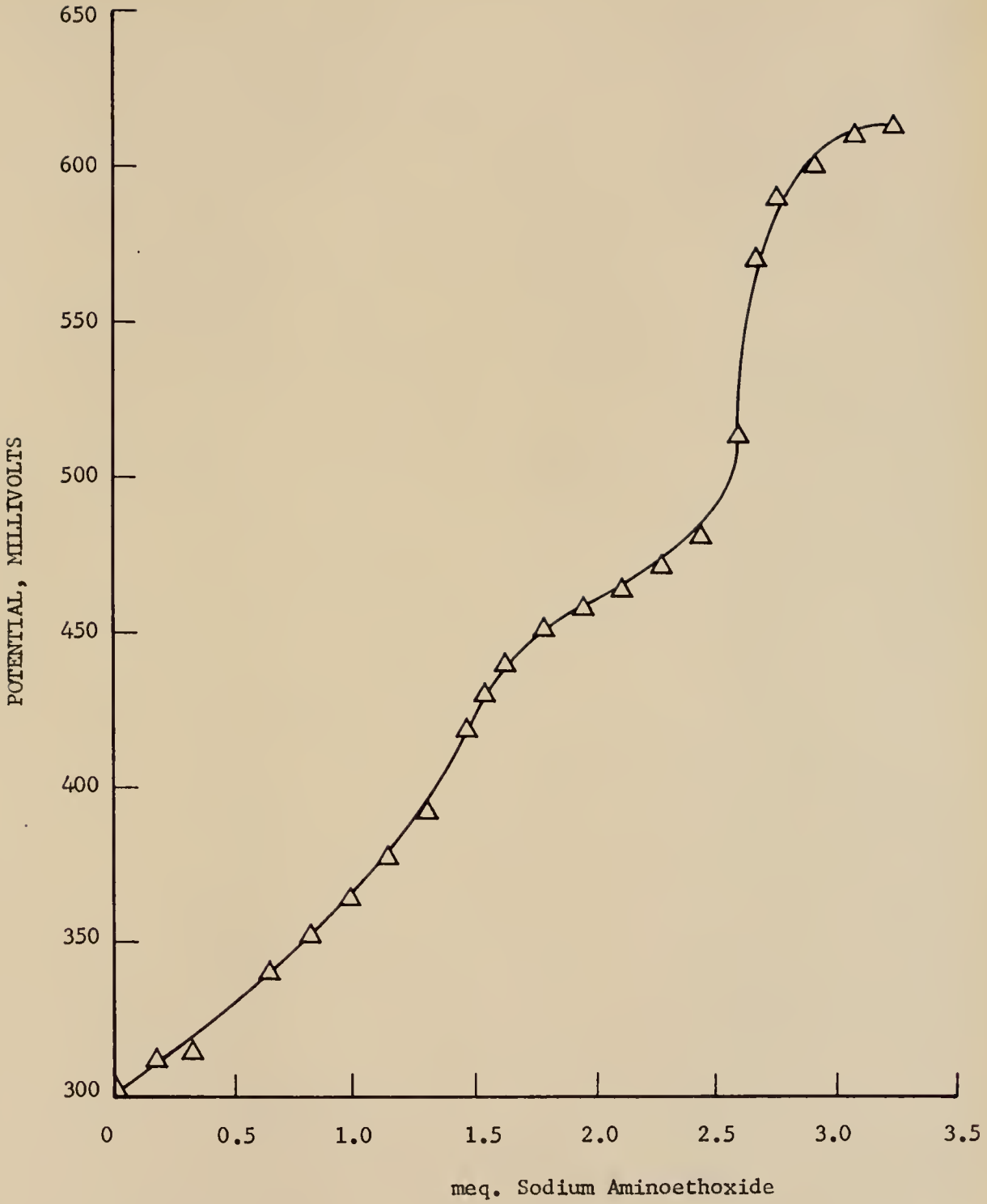


Figure 33. Potentiometric Titration of p-Hydroxybenzoic Acid in Ethylenediamine

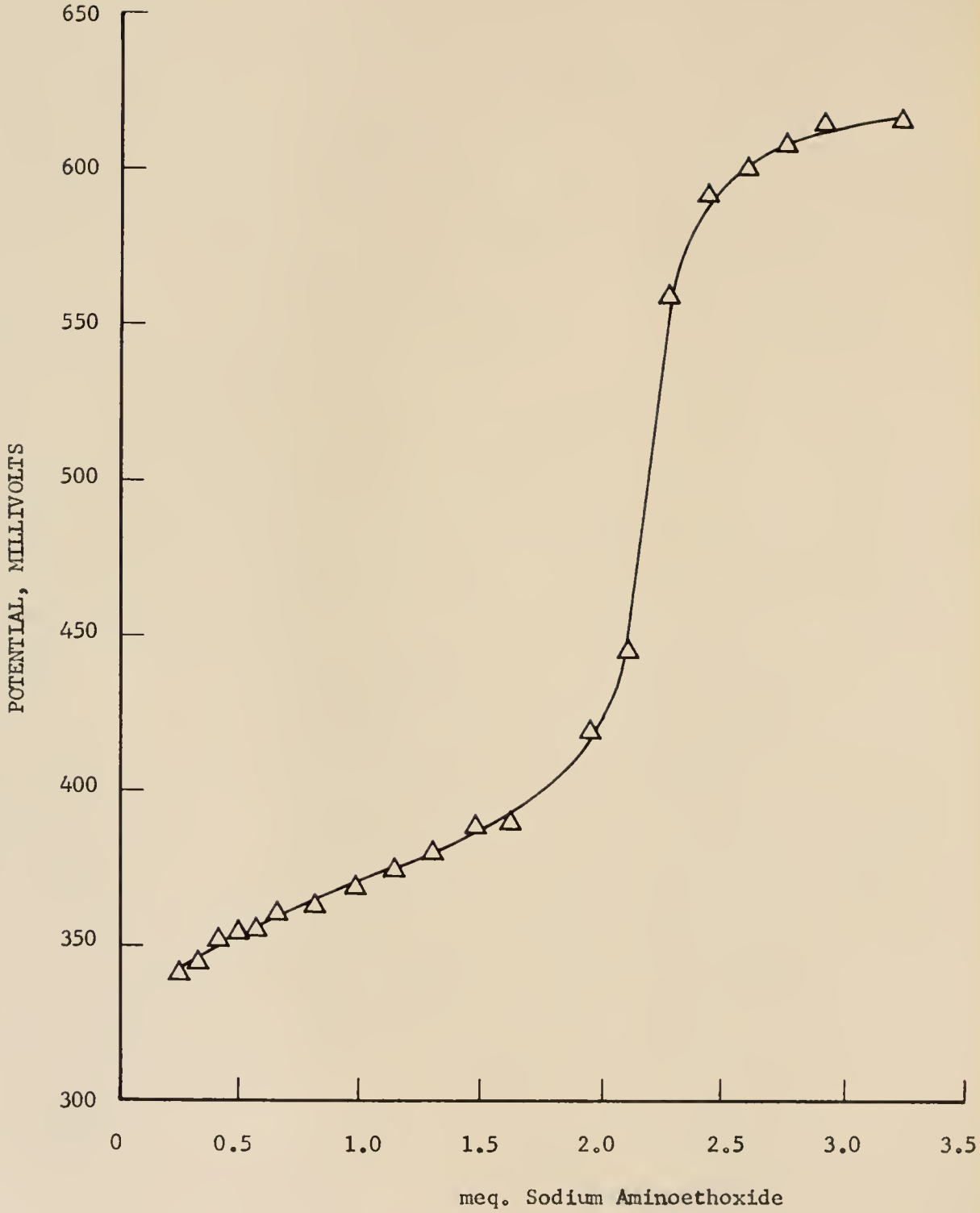


Figure 34. Potentiometric Titration of Fulvic Acid Fraction of Water B in Ethylenediamine

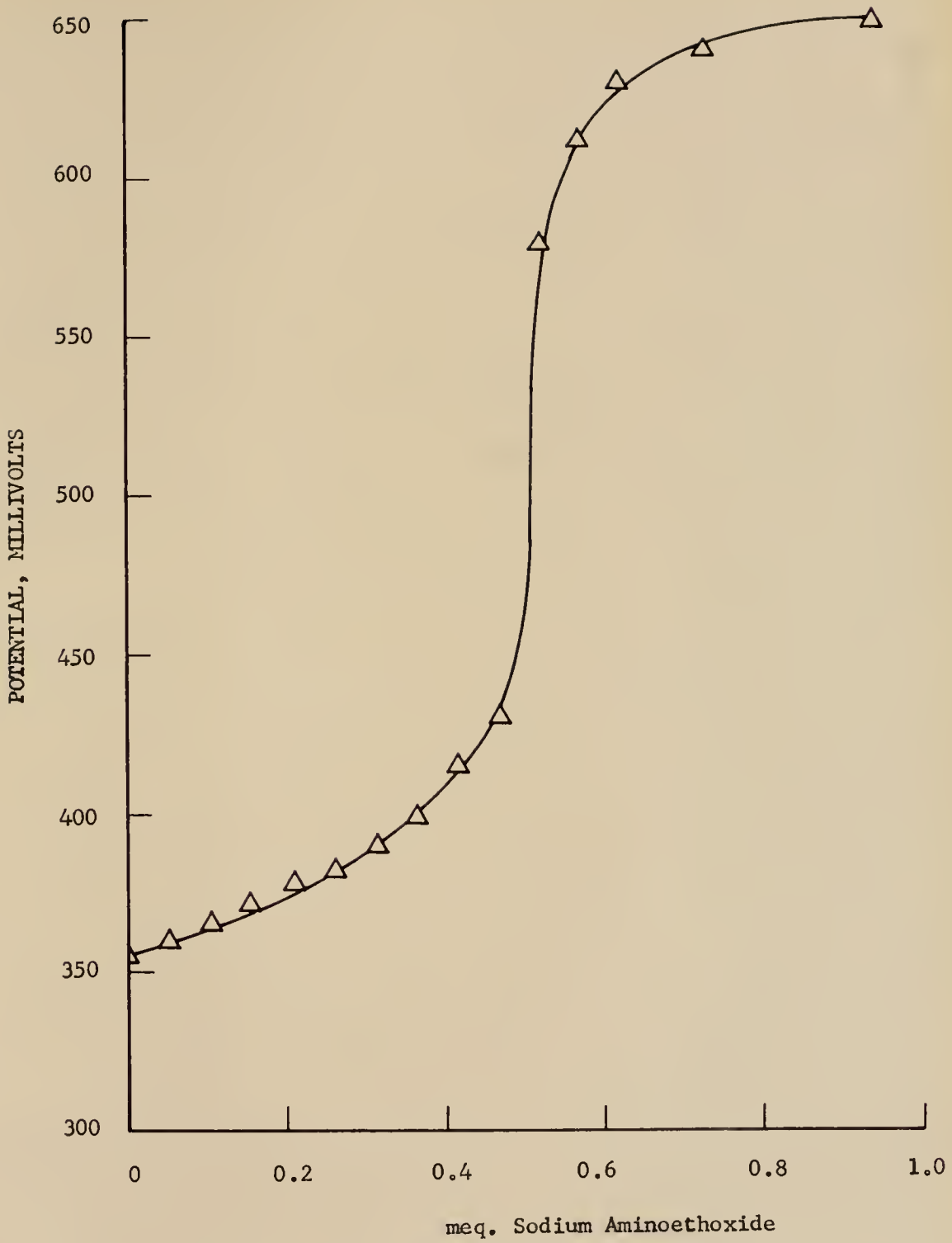


Figure 35. Potentiometric Titration of Fulvic Acid Fraction of Water I in Ethylenediamine

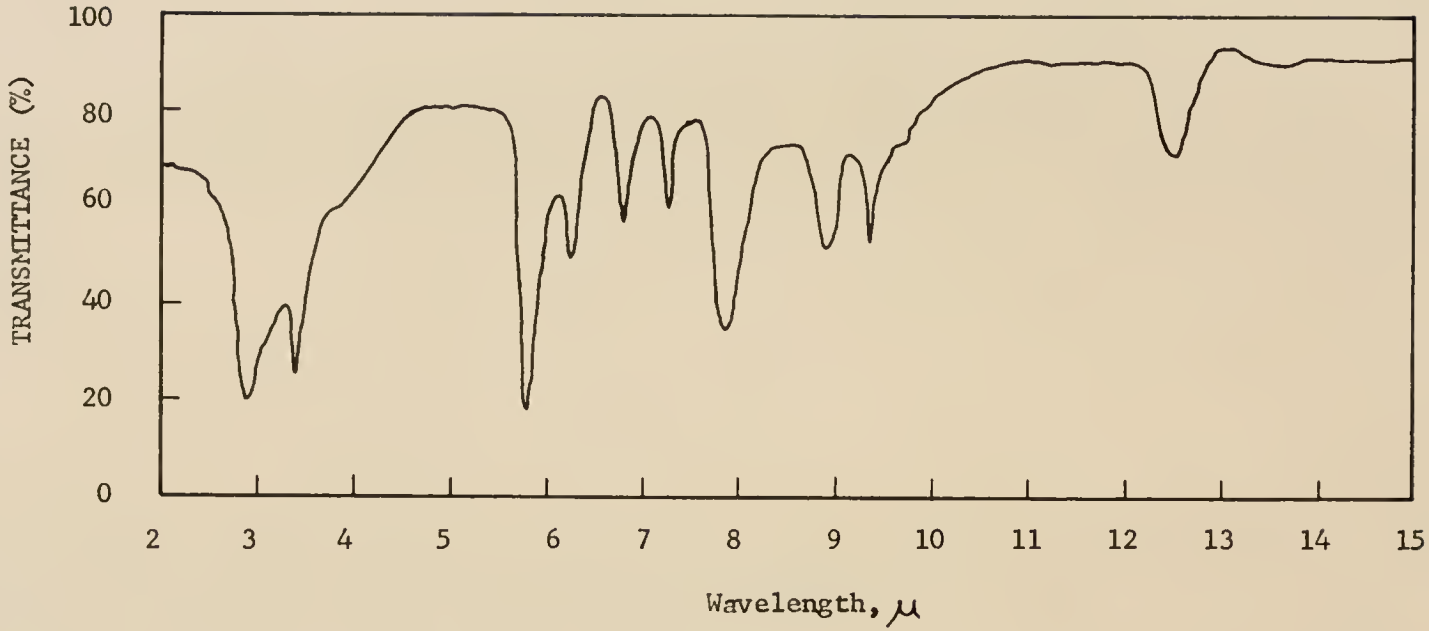


Figure 36. Infra-Red Absorption Spectrum of Chlorine Oxidation Product from Water A

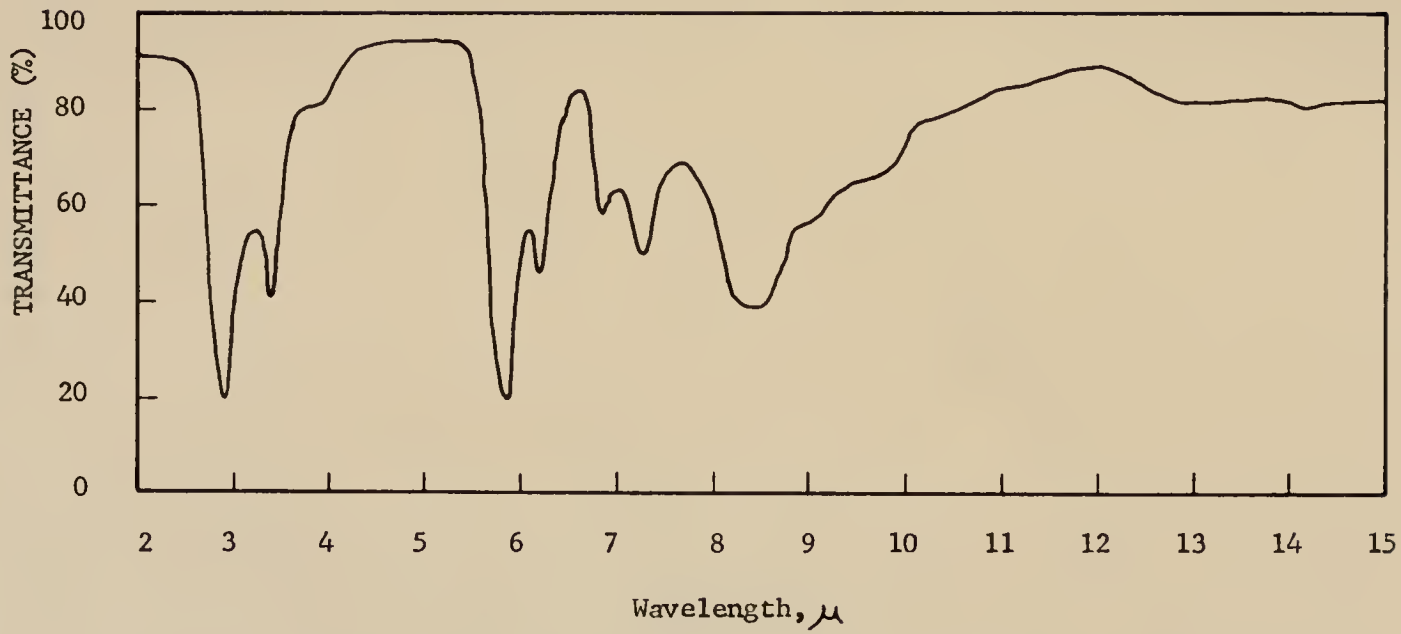


Figure 37. Infra-Red Absorption Spectrum of Chlorine Oxidation Product Extracted from Miami Tap Water

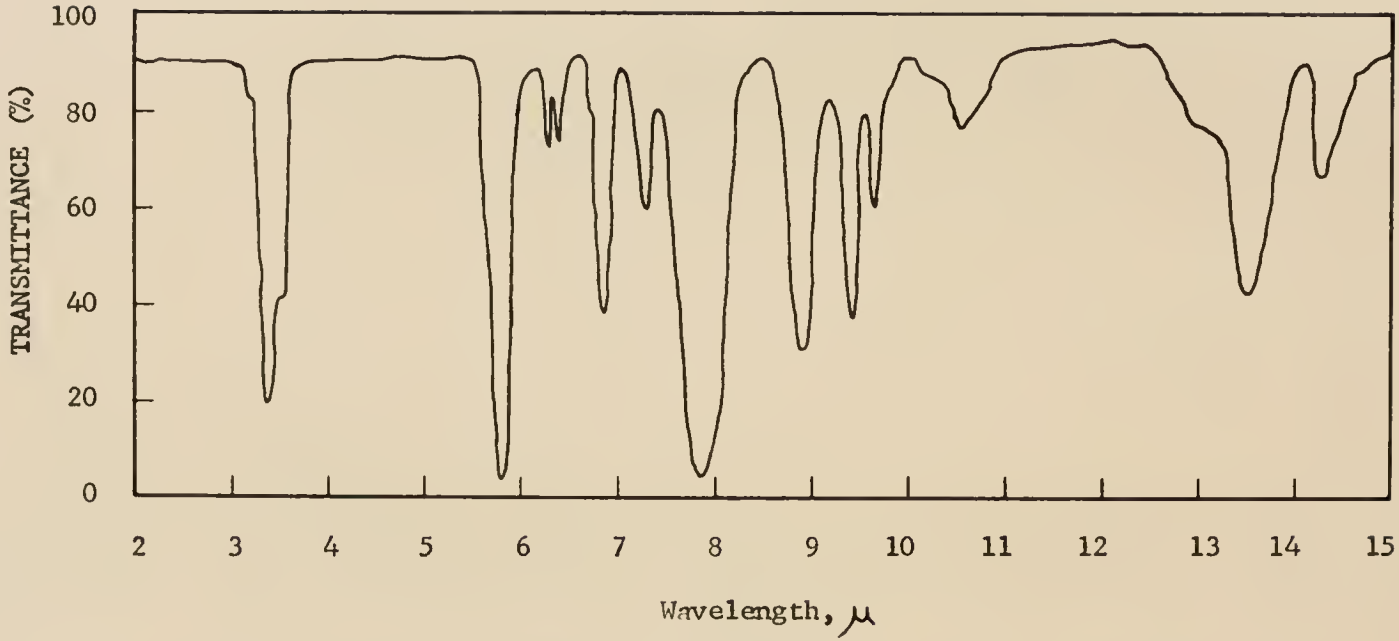


Figure 38. Infra-Red Absorption Spectrum of a Fulvic Acid Oxidation Product

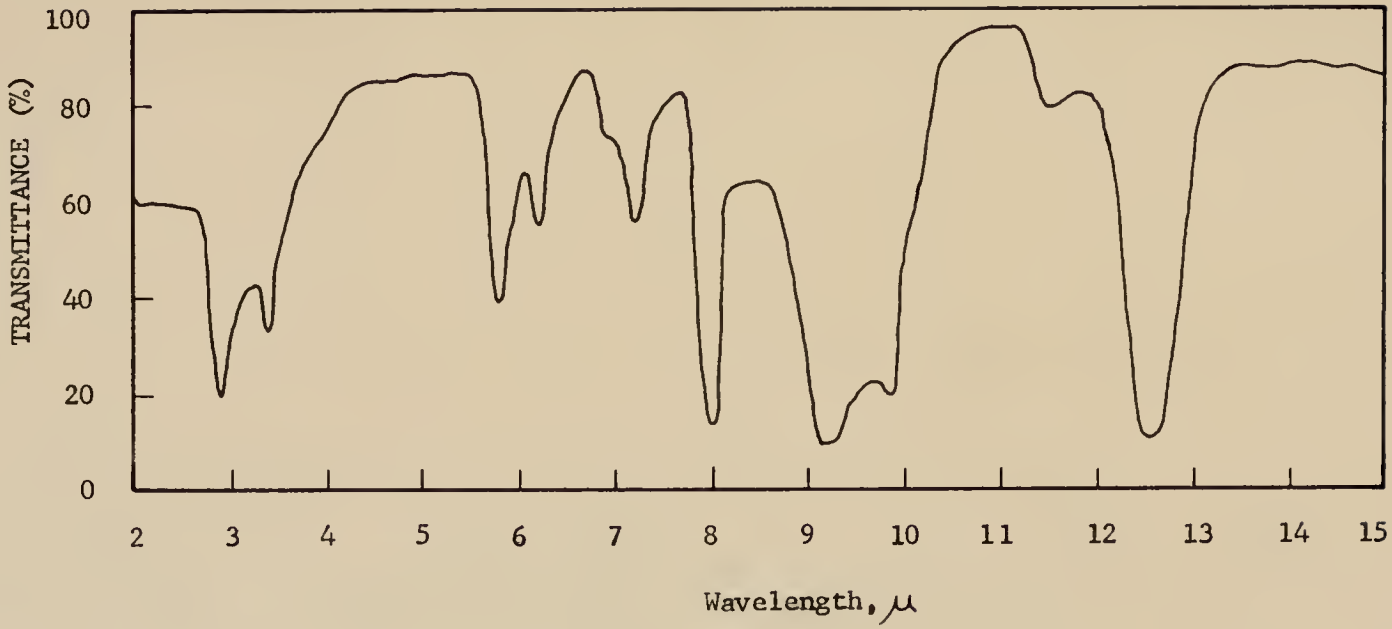


Figure 39. Infra-Red Absorption Spectrum of a Fulvic Acid Oxidation Product

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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